10AU

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C07H 21/04, C12N 15/00, C12P 21/00, C07K 14/00, A61K 39/395, 38/00, 31/00

A1

(11) International Publication Number: WO 97/34911

(43) International Publication Date: 25 September 1997 (25.09.97)

(21) International Application Number: PCT/US96/16966

(22) International Filing Date: 31 October 1996 (31.10.96)

(30) Priority Data:
60/013,923 22 March 1996 (22.03.96) US

(71) Applicant (for all designated States except US): HUMAN GENOME SCIENCES, INC. [US/US]; 9410 Key West Avenue, Rockville, MD 20850 (US).

(72) Inventors; and
(75) Inventors/Applicants (for US only): EBNER, Reinhard [DE/US]; 9906 Shelbourne Terrace #316, Gaithersburg, MD 20878 (US). RUBEN, Steven, M. [US/US]; 18528 Heritage Hills Drive, Olney, MD 20832 (US). YU, Guo-Liang [CN/US]; 13524 Straw Bale Lane, Darnestown, MD 20878 (US).

(74) Agents: GOLDSTEIN, Jorge, A. et al.; Sterne, Kessler, Goldstein & Fox P.L.L.C., Suite 600, 1100 New York Avenue, N.W., Washington, DC 20005-3934 (US).

(81) Designated States: AM, AU, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, IL, JP, KG, KP, KR, KZ, LT, LV, MD, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TM, TR, UA, US, UZ, VN, Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: APOPTOSIS INDUCING MOLECULE II

(57) Abstract

The present invention relates to a novel member of the TNF-Ligand superfamily, Apoptosis Inducing Molecule II (AIM II). In particular, isolated nucleic acid molecules are provided encoding the human AIM II protein. AIM II polypeptides are also provided as are vectors, host cells and recombinant methods for producing the same. The invention further relates to screening methods for identifying agonists and antagonists of AIM II activity. Also provided are therapeutic methods for treating lymphadenopathy, autoimmune disease, graft versus host disease, and to inhibit neoplasia, such as tumor cell growth.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania
AM	Armenia
AT	Austria
AU	Australia
AZ	Azerbaijan
BA	Bosnia and Herzegovina
BB	Barbados
BE	Belgium
BF	Burkina Faso
BG	Bulgaria
BJ	Benin
BR	Brazil
BY	Belarus
CA	Canada
CF	Central African Republic
CG	Congo
CH	Switzerland
CI	Côte d'Ivoire
CM	Cameroon
CN	China
CU	Cuba
CZ	Czech Republic
DE	Germany
DK	Denmark
EE	Estonia
ES	Spain
FI	Finland
FR	France
GA	Gabon
GB	United Kingdom
GE	Georgia
GH	Ghana
GN	Guinea
GR	Greece
HU	Hungary
IE	Ireland
IL	Israel
IS	Iceland
IT	Italy
JP	Japan
KE	Kenya
KG	Kyrgyzstan
KP	Democratic People's Republic of Korea
KR	Republic of Korea
KZ	Kazakstan
LC	Saint Lucia
LI	Liechtenstein
LK	Sri Lanka
LR	Liberia
LS	Lesotho
LT	Lithuania
LU	Luxembourg
LV	Latvia
MC	Monaco
MD	Republic of Moldova
MG	Madagascar
MK	The former Yugoslav Republic of Macedonia
ML	Mali
MN	Mongolia
MR	Mauritania
MW	Malawi
MX	Mexico
NE	Niger
NL	Netherlands
NO	Norway
NZ	New Zealand
PL	Poland
PT	Portugal
RO	Romania
RU	Russian Federation
SD	Sudan
SE	Sweden
SG	Singapore
SI	Slovenia
SK	Slovakia
SN	Senegal
SZ	Swaziland
TD	Chad
TG	Togo
TJ	Tajikistan
TM	Turkmenistan
TR	Turkey
TT	Trinidad and Tobago
UA	Ukraine
UG	Uganda
US	United States of America
UZ	Uzbekistan
VN	Viet Nam
YU	Yugoslavia
ZW	Zimbabwe

Apoptosis Inducing Molecule II

Background of the Invention

Field of the Invention

The present invention relates to a novel member of the TNF-Ligand superfamily. More specifically, isolated nucleic acid molecules are provided encoding a human Apoptosis Inducing Molecule II (AIM II). AIM II polypeptides are also provided, as are vectors, host cells and recombinant methods for producing the same. The invention further relates to screening methods for identifying agonists and antagonists of AIM II activity. Also provided are therapeutic methods for treating lymphadenopathy, autoimmune disease, graft versus host disease, and to inhibit neoplasia, such as tumor cell growth.

Related Art

Human tumor necrosis factors α (TNF-α) and β (TNF-β, or lymphotoxin) are related members of a broad class of polypeptide mediators, which includes the interferons, interleukins and growth factors, collectively called cytokines (Beutler, B. and Cerami, A., *Annu. Ret,. Immunol.*, 7:625-655 (1989)).

Tumor necrosis factor (TNF-α and TNF-β) was originally discovered as a result of its anti-tumor activity, however, now it is recognized as a pleiotropic cytokine capable of numerous biological activities including apoptosis of some transformed cell lines, mediation of cell activation and proliferation and also as playing important roles in immune regulation and inflammation.

To date, known members of the TNF-ligand superfamily include TNF-α, TNF-β (lymphotoxin-α). LT-β, OX40L, Fas ligand, CD30L, CD27L, CD40L and 4-IBBL. The ligands of the TNF ligand superfamily are acidic, TNF-like molecules with approximately 20% sequence homology in the extracellular domains (range, 12%-36%) and exist mainly as membrane-bound forms with the biologically active form being a trimeric/multimeric complex. Soluble forms of

the TNF ligand superfamily have only been identified so far for TNF, LTα, and Fas ligand (for a general review, see Gruss, H. and Dower, S.K., *Blood, 85(12)*:3378-3404 (1995)), which is hereby incorporated by reference in its entirety.

These proteins are involved in regulation of cell proliferation, activation, and differentiation, including control of cell survival or death by apoptosis or cytotoxicity (Armitage, R.J., *Curr. Opin. Immunol. 6*:407 (1994) and Smith, C.A., *Cell 75*:959 (1994)).

Mammalian development is dependent on both the proliferation and differentiation of cells as well as programmed cell death which occurs through apoptosis (Walker, *et al.*, *Methods Achiev. Exp. Pathol. 13*:18 (1988). Apoptosis plays a critical role in the destruction of immune thymocytes that recognize self antigens. Failure of this normal elimination process may play a role in autoimmune diseases (Gammon *et al., Immunology Today 12*:193 (1991)).

Itoh *et al.* (*Cell 66*:233 (1991)) described a cell surface antigen, Fas/CD95 that mediates apoptosis and is involved in clonal deletion of T-cells. Fas is expressed in activated T-cells, B-cells, neutrophils and in thymus, liver, heart and lung and ovary in adult mice (Watanabe-Fukunaga *et al., J. Immunolo. 148*:1274 (1992)) in addition to activated T-cells, B-cells, neutorophils. In experiments where a monoclonal Ab to Fas is cross-linked to Fas, apoptosis is induced (Yonehara *et al., J. Exp. Med. 169*:1747 (1989); Trauth *et al., Science 245*:301 (1989)). In addition, there is an example where binding of a monoclonal Ab to Fas may stimulate T-cells under certain conditions (Alderson *et al., J. Exp. Med. 178*:2231 (1993)).

Fas antigen is a cell surface protein of relative MW of 45 Kd. Both human and murine genes for Fas have been cloned by Watanabe-Fukunaga *et al.*, (*J. Immunol. 148*:1274 (1992)) and Itoh *et al.* (*Cell 66*:233 (1991)). The proteins encoded by these genes are both transmembrane proteins with structural homology to the Nerve Growth Factor/Tumor Necrosis Factor receptor

superfamily, which includes two TNF receptors, the low affinity Nerve Growth Factor receptor and the LT_β receptor CD40, CD27, CD30, and OX40.

Recently the Fas ligand has been described (Suda *et al., Cell 75*:II69 (1993)). The amino acid sequence indicates that Fas ligand is a type II transmembrane protein belonging to the TNF family. Fas ligand is expressed in splenocytes and thymocytes. The purified Fas ligand has a MW of 40 kd.

Recently, it has been demonstrated that Fas/Fas ligand interactions are required for apoptosis following the activation of T-cells (Ju *et al., Nature 373*:444 (1995); Brunner *et al., Nature 373*:441 (1995)). Activation of T-cells induces both proteins on the cell surface. Subsequent interaction between the ligand and receptor results in apoptosis of the cells. This supports the possible regulatory role for apoptosis induced by Fas/Fas ligand interaction during normal immune responses.

The polypeptide of the present invention has been identified as a novel member of the TNF ligand super-family based on structural and biological similarities.

Clearly, there is a need for factors that regulate activation, and differentiation of normal and abnormal cells. There is a need, therefore, for identification and characterization of such factors that modulate activation and differentiation of cells, both normally and in disease states. In particular, there is a need to isolate and characterize additional Fas ligands that control apoptosis ofr the treatment of autoimmune disease, graft versus host disease and lymphadenopathy.

Summary of the Invention

The present invention provides isolated nucleic acid molecules comprising a polynucleotide encoding the AIM II polypeptide having the amino acid sequence shown in Figures 1A-C (SEQ ID NO:2) or the amino acid sequence

encoded by the cDNA clone deposited in a bacterial host as ATCC Deposit Number 97689 on August 22, 1996.

The present invention also relates to recombinant vectors, which include the isolated nucleic acid molecules of the present invention, and to host cells containing the recombinant vectors, as well as to methods of making such vectors and host cells and for using them for production of AIM II polypeptides or peptides by recombinant techniques.

The invention further provides an isolated AIM II polypeptide having an amino acid sequence encoded by a polynucleotide described herein.

As used herein the term "AIM II" polypeptide includes membrane-bound proteins (comprising a cytoplasmic domain, a transmembrane domain, and an extracellular domain) as well as truncated proteins that retain the AIM II polypeptide activity. In one embodiment, soluble AIM II polypeptides comprise all or part of the extracellular domain of an AIM II protein, but lack the transmsmbrane region that would cause retention of the polypeptide on a cell membrane. Soluble AIM II may also include part of the transmembrane region or part of the cytoplasmic domain or other sequences, provided that the soluble AIM II protein is capable of being secreted. A heterologous signal peptide can be fused to the N-terminus of the soluble AIM II polypeptide such that the soluble AIM II polypeptide is secreted upon expression.

The invention also provides for AIM II polypeptides, particularly human AIM-II polypeptides, which may be employed to treat lymphadenopathy, autoimmune disease, graft versus host disease, which may be used to stimulate peripheral tolerance, destroy some transformed cell lines, mediate cell activation and proliferation and are functionally linked as primary mediators of immune regulation and inflammatory response.

The invention further provides compositions comprising an AIM II polynucleotide or an AIM II polypeptide for administration to cells *in vitro*, to cells *ex vivo* and to cells *in vivo*. or to a multicellular organism. In certain particularly preferred embodiments of this aspect of the invention, the

compositions comprise an AIM II polynucleotide for expression of an AIM II polypeptide in a host organism for treatment of disease. Particularly preferred in this regard is expression in a human patient for treatment of a dysfunction associated with aberrant endogenous activity of an AIM II.

. The present invention also provides a screening method for identifying compounds capable of enhancing or inhibiting a cellular response induced by AIM II, which involves contacting cells which express AIM II with the candidate compound, assaying a cellular response, and comparing the cellular response to a standard cellular response, the standard being assyed when contact is made in absence of the candidate compound; whereby, an increased cellular response over the standard indicates that the compound is an agonist and a decreased cellular response over the standard indicates that the compound is an antagonist.

In another aspect, a screening assay for AIM II agonists and antagonists is provided. The antagonists may be employed to prevent septic shock, inflammation, cerebral malaria, activation of the HIV virus, graft-host rejection, bone resorption, rheumatoid arthritis and cachexia (wasting or malnutrition).

An additional aspect of the invention is related to a method for treating an individual in need of an increased level of AIM II activity in the body comprising administering to such an indivdual a composition comprising a therapeutically effective amount of an isolated AIM II polypeptide of the invention or an agonist thereof.

A still further aspect of the invention is related to a method for treating an individual in need of a decreased level of AIM II activity in the body comprising, administering to such an individual a composition comprising a therapeutically effective amount of an AIM II antagonist.

Brief Description of the Figures

Figures 1A-C show the nucleotide (SEQ ID NO:1) and deduced amino acid (SEQ ID NO:2) sequences of AIM II. The protein has a deduced molecular

weight of about 26.4 kDa. The predicted Transmembrane Domain of the AIM II protein is underlined.

Figures 2A-F show the regions of similarity between the amino acid sequences of the AIM II protein and human TNF-α (SEQ ID NO: 3), human TNF-β (SEQ ID NO:4), human lymphotoxin (SEQ ID NO:5) and human Fas Ligand (SEQ ID NO:6).

Figures 3A-F show an analysis of the AIM II amino acid sequence. Alpha, beta, turn and coil regions; hydrophilicity and hydrophobicity; amphipathic regions; flexible regions; antigenic index and surface probability are shown. In the "Antigenic Index - Jameson-Wolf" graph, about amino acid residues 13-20, 23-36, 69-79, 85-94, 167-178, 184-196, 221-233 in Figures 1A-C correspond to the shown highly antigenic regions of the AIM II protein.

Detailed Description

The present invention provides isolated nucleic acid molecules comprising a polynucleotide encoding an AIM II polypeptide having the amino acid sequence shown in Figures 1A-C (SEQ ID NO:2), which was determined by sequencing a cloned cDNA. The AIM II protein of the present invention shares sequence homology with human TNF-α (SEQ ID NO: 3), human TNF-β (SEQ ID NO:4), human lymphotoxin (SEQ ID NO:5) and human Fas Ligand (SEQ ID NO:6) (Figures 2A-F). The nucleotide sequence shown in Figures 1A-C (SEQ ID NO:1) were obtained by sequencing the a cDNA clone, which was deposited on August 22, 1996 at the American Type Culture Collection, 12301 Park Lawn Drive, Rockville. Maryland 20852, and given accession number 97689. The deposited clone is contained in the pBluescript SK(-) plasmid (Stratagene, La Jolla, CA).

Nucleic Acid Molecules

Unless otherwise indicated, all nucleotide sequences determined by sequencing a DNA molecule herein were determined using an automated DNA sequencer (such as the Model 373 from Applied Biosystems, Inc.), and all amino acid sequences of polypeptides encoded by DNA molecules determined herein were predicted by translation of a DNA sequence determined as above. Therefore, as is known in the art for any DNA sequence determined by this automated approach, any nucleotide sequence determined herein may contain some errors. Nucleotide sequences determined by automation are typically at least about 90% identical, more typically at least about 95% to at least about 99.9% identical to the actual nucleotide sequence of the sequenced DNA molecule. The actual sequence can be more precisely determined by other approaches including manual DNA sequencing methods well known in the art. As is also known in the art, a single insertion or deletion in a determined nucleotide sequence compared to the actual sequence will cause a frame shift in translation of the nucleotide sequence such that the predicted amino acid sequence encoded by a determined nucleotide sequence will be completely different from the amino acid sequence actually encoded by the sequenced DNA molecule, beginning at the point of such an insertion or deletion.

Using the information provided herein, such as the nucleotide sequence in Figures 1A-C, a nucleic acid molecule of the present invention encoding an AIM II polypeptide may be obtained using standard cloning and screening procedures, such as those for cloning cDNAs using mRNA as starting material. Illustrative of the invention, the nucleic acid molecule described in Figures 1A-C (SEQ ID NO:1) was discovered in a cDNA library derived from human macrophage ox LDL (HMCCB64). The gene was also identified in cDNA libraries from activated T-cells (HT4CC72). The determined nucleotide sequence of the AIM II cDNA of Figures 1A-C (SEQ ID NO:1) contains an open reading frame encoding a protein of 240 amino acid residues, with an initiation codon at

positions 49-51 of the nucleotide sequence in Figures 1A-C (SEQ ID NO:1), an extracellular domain comprising amino acid residues from about 60 to about 240 in Figures 1A-C (SEQ ID NO:2), a transmembrane domain comprising amino acid residues from about 37 to about 59 in Figures 1A-C (SEQ ID NO:2), a intracellular domain comprising amino acid residues from about 1 to about 36 in Figures 1A-C (SEQ ID NO:2) and a deduced molecular weight of about 26.4 kDa. The AIM II protein shown in Figures 1A-C (SEQ ID NO:2) is about 27% identical and about 51% similar to the amino acid sequence of human Fas Ligand(Figures 2A-F) and is about 26% identical and about 47% similar to the amino acid sequence of human TNF-α (Figures 2A-F).

As one of ordinary skill would appreciate, due to the possibilities of sequencing errors discussed above, the predicted AIM II polypeptide encoded by the deposited cDNA comprises about 240 amino acids, but may be anywhere in the range of 230-250 amino acids. It will further be appreciated that, depending on the criteria used, concerning the exact "address" of the extracelluar, intracelluar and transmembrane domains of the AIMII polypeptide differ slightly. For example, the exact location of the AIM II extracellular domain in Figures 1A-C [SEQ ID NO:2] may vary slightly (e.g., the address may "shift" by about 1 to 5 residues) depending on the criteria used to define the domain.

As indicated, nucleic acid molecules of the present invention may be in the form of RNA, such as mRNA, or in the form of DNA, including, for instance, cDNA and genomic DNA obtained by cloning or produced synthetically. The DNA may be double-stranded or single-stranded. Single-stranded DNA or RNA may be the coding strand, also known as the sense strand, or it may be the non-coding strand, also referred to as the anti-sense strand.

By "isolated" nucleic acid molecule(s) is intended a nucleic acid molecule, DNA or RNA, which has been removed from its native environment For example, recombinant DNA molecules contained in a vector are considered isolated for the purposes of the present invention. Further examples of isolated DNA molecules include recombinant DNA molecules maintained in heterologous

host cells or purified (partially or substantially) DNA molecules in solution. Isolated RNA molecules include *in vivo* or *in vitro* RNA transcripts of the DNA molecules of the present invention. Isolated nucleic acid molecules according to the present invention further include such molecules produced synthetically.

Isolated nucleic acid molecules of the present invention include DNA molecules comprising an open reading frame (ORF) shown in Figures 1A-C (SEQ ID NO:1); DNA molecules comprising the coding sequence for the AIM II protein shown in Figures 1A-C (SEQ ID NO:2); and DNA molecules which comprise a sequence substantially different from those described above but which, due to the degeneracy of the genetic code, still encode the AIM II protein. Of course, the genetic code is well known in the art. Thus, it would be routine for one skilled in the art to generate such degenerate variants.

In another aspect, the invention provides isolated nucleic acid molecules encoding the AIM II polypeptide having an amino acid sequence encoded by the cDNA clone contained in the plasmid deposited as ATCC Deposit No. 97689 on August 22, 1996. Preferably, this nucleic acid molecule will encode the polypeptide encoded by the above-described deposited cDNA clone. The invention further provides an isolated nucleic acid molecule having the nucleotide sequence shown in Figures 1A-C (SEQ ID NO:1) or the nucleotide sequence of the AIM II cDNA contained in the above-described deposited clone, or a nucleic acid molecule having a sequence complementary to one of the above sequences. Such isolated molecules, particularly DNA molecules, are useful as probes for gene mapping, by *in situ* hybridization with chromosomes, and for detecting expression of the AIM II gene in human tissue, for instance, by Northern blot analysis.

The present invention is further directed to fragments of the isolated nucleic acid molecules described herein. By a fragment of an isolated nucleic acid molecule having the nucleotide sequence of the deposited cDNA or the nucleotide sequence shown in Figures 1A-C (SEQ ID NO:1) is intended fragments at least about 15 nt. and more preferably at least about 20 nt, still more

preferably at least about 30 nt, and even more preferably, at least about 40 nt in length which are useful as diagnostic probes and primers as discussed herein. Of course, larger fragments 50-1500 nt in length are also useful according to the present invention as are fragments corresponding to most, if not all, of the nucleotide sequence of the deposited cDNA or as shown in Figures 1A-C (SEQ ID NO:1). By a fragment at least 20 nt in length, for example, is intended fragments which include 20 or more contiguous bases from the nucleotide sequence of the deposited cDNA or the nucleotide sequence as shown in Figures 1A-C (SEQ ID NO:1).

Preferred nucleic acid fragments of the present invention include nucleic acid molecules encoding epitope-bearing portions of the AIM II protein. In particular, such nucleic acid fragments of the present invention include nucleic acid molecules encoding: a polypeptide comprising amino acid residues from about 13 to about 20 in Figures 1A-C (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 23 to about 36 in Figures 1A-C (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 69 to about 79 in Figures 1A-C (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 85 to about 94 in Figures 1A-C (SEQ ID NO:2);a polypeptide comprising amino acid residues from about 167 to about 178 in Figures 1A-C (SEQ ID NO:2);a polypeptide comprising amino acid residues from about 184 to about 196 in Figures 1A-C (SEQ ID NO:2); and a polypeptide comprising amino acid residues from about 221 to about 233 in Figures 1A-C (SEQ ID NO:2). The inventors have determined that the above polypeptide fragments are antigenic regions of the AIM II protein. Methods for determining other such epitope-bearing portions of the AIM II protein are described in detail below.

AIM-II polynucleotides may be used in accordance with the present invention for a variety of applications, particularly those that make use of the chemical and biological properties of the AIM-II. Among these applications in

autoimmune disease and aberrant cellular proliferation. Additional applications relate to diagonsis and to treatment of disorders of cells, tissues, and organisms.

This invention is also related to the use of the AIM-II polynucleotides to detect complementary polynucleotides such as, for example, as a diagnostic reagent. Detection of a mutated form of an AIM-II associated with a dysfunction will provide a diagnostic tool that can add or define a diagnosis of a disease or susceptibility to disease which reults from under-expression, over-expression or altered expression of AIM-II, such as, for example, autoimmune diseases. The polynucleotide encoding the AIM-II may also be employed as a diagnostic marker for expression of the polypeptide of the present invention since the gene is found in many tumor cell lines including pancreatic tumor, testes tumor, endometrial tumor and T-cell lymphoma.

In another aspect, the invention provides an isolated nucleic acid molecule comprising a polynucleotide which hybridizes under stringent hybridization conditions to a portion of the polynucleotide in a nucleic acid molecule of the invention described above, for instance, the cDNA clone contained in ATCC Deposit 97689. By "stringent hybridization conditions" is intended overnight incubation at 42°C in a solution comprising: 50% formamide, 5x SSC (150 mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 g/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65°C.

By a polynucleotide which hybridizes to a "portion" of a polynucleotide is intended a polynucleotide (either DNA or RNA) hybridizing to at least about 15 nucleotides (nt), and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably about 30-70 nt of the reference polynucleotide. These are useful as diagnostic probes and primers as discussed above and in more detail below.

By a portion of a polynucleotide of "at least 20 nt in length," for example, is intended 20 or more contiguous nucleotides from the nucleotide sequence of

the reference polynucleotide (e.g., the deposited cDNA or the nucleotide sequence as shown in Figures 1A-C (SEQ ID NO:1)).

Of course, a polynucleotide which hybridizes only to a poly A sequence (such as the 3′ terminal poly(A) tract of the AIM II cDNA shown in Figures 1A-C (SEQ ID NO:1)), or to a complementary stretch of T (or U) resides, would not be included in a polynucleotide of the invention used to hybridize to a portion of a nucleic acid of the invention, since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone).

As indicated, nucleic acid molecules of the present invention which encode an AIM II polypeptide may include, but are not limited to those encoding the amino acid sequence of the polypeptide, by itself; the coding sequence for the polypeptide and additional sequences, such as those encoding a leader or secretory sequence, such as a pre-, or pro- or prepro- protein sequence; the coding sequence of the polypeptide, with or without the aforementioned additional coding sequences, together with additional, non-coding sequences, including for example, but not limited to introns and non-coding 5′ and 3′ sequences, such as the transcribed, non-translated sequences that play a role in transcription, mRNA processing, including splicing and polyadenylation signals, for example - ribosome binding and stability of mRNA; an additional coding sequence which codes for additional amino acids, such as those which provide additional functionalities. Thus, the sequence encoding the polypeptide may be fused to a marker sequence, such as a sequence encoding a peptide which facilitates purification of the fused polypeptide. In certain preferred embodiments of this aspect of the invention, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (Qiagen, Inc.), among others, many of which are commercially available. As described in Gentz *et al., Proc. Natl. Acad. Sci. USA 86*:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. The "HA" tag is another peptide useful for purification which corresponds to an epitope derived from the influenza

hemagglutinin protein, which has been described by Wilson *et al., Cell 37*: 767 (1984). As discussed below, other such fusion proteins include the AIM II fused to Fc at the N- or C-terminus.

Nucleic acid molecules according to the present invention further include those encoding the full-length AIM-II polypeptide lacking the N-terminal methonine.

The present invention further relates to variants of the nucleic acid molecules of the present invention, which encode portions, analogs or derivatives of the AIM II protein. Variants may occur naturally, such as a natural allelic variant. By an "allelic variant" is intended one of several alternate forms of a gene occupying a given locus on a chromosome of an organism. *Genes II*, Lewin, B., ed., John Wiley & Sons, New York (1985). Non-naturally occurring variants may be produced using art-known mutagenesis techniques.

Such variants include those produced by nucleotide substitutions, deletions or additions which may involve one or more nucleotides. The variants may be altered in coding regoins, non-coding regions, or both. Alterations in the coding regions may produce conservative or non-conservative amino acid substitutions, deletions or additions. Especially preferred among these are silent substitutions, additions and deletions, which do not alter the properties and activities of the AIM II protein or portions thereof. Also especially preferred in this regard are conservative substitutions.

Further embodiments of the invention include isolated nucleic acid molecules comprising a polynucleotide having a nucleotide sequence at least 90% identical, and more preferably at least 95%, 96%, 97%, 98% or 99% identical to (a) a nucleotide sequence encoding the AIM II polypeptide having the complete amino acid sequence in Figures 1A-C (SEQ ID NO:2); (b) a nucleotide sequence encoding the AIM II polypeptide having the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97689; (c) a nucleotide sequence encoding the AIM II polypeptide extracellular domain; (d) a nucleotide sequence encoding the AIM II polypeptide transmembrane domain;

(e) a nucleotide sequence encoding the AIM II polypeptide intracellular domain; (f) a nucleotide sequence encoding a soluble AIM II polypeptide having the extracellular and intracellular domains but lacking the transmembrane domain; and (g) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e) or (f) above.

By a polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence encoding an AIM II polypeptide is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence encoding the AIM II polypeptide. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations of the reference sequence may occur at the 5′ or 3′ terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular nucleic acid molecule is at least 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the nucleotide sequence shown in Figures 1A-C or to the nucleotides sequence of the deposited cDNA clone can be determined conventionally using known computer programs such as the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711. Bestfit uses the local homology algorithm of Smith and Waterman, *Advances in Applied Mathematics 2*: 482-489 (1981), to find the best segment of homology between two sequences. When using Bestfit or any other sequence alignment program to determine whether a particular sequence is,

for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference nucleotide sequence and that gaps in homology of up to 5% of the total number of nucleotides in the reference sequence are allowed.

The present application is directed to nucleic acid molecules at least 90%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequence shown in Figures 1A-C (SEQ ID NO:1) or to the nucleic acid sequence of the deposited cDNA, irrespective of whether they encode a polypeptide having AIM II activity. This is because even where a particular nucleic acid molecule does not encode a polypeptide having AIM II activity, one of skill in the art would still know how to use the nucleic acid molecule, for instance, as a hybridization probe or a polymerase chain reaction (PCR) primer. Uses of the nucleic acid molecules of the present invention that do not encode a polypeptide having AIM II activity include, *inter alia,* (1) isolating the AIM II gene or allelic variants thereof in a cDNA library; (2) *in situ* hybridization (e.g., "FISH") to metaphase chromosomal spreads to provide precise chromosomal location of the AIM II gene, as described in Verma *et al., Human Chromosomes: A Manual of Basic Techniques*, Pergamon Press, New York (1988); and Northern Blot analysis for detecting AIM II mRNA expression in specific tissues.

Preferred, however, are nucleic acid molecules having sequences at least 90%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequence shown in Figures 1A-C (SEQ ID NO:1) or to the nucleic acid sequence of the deposited cDNA which do, in fact, encode a polypeptide having AIM II protein activity. By "a polypeptide having AIM II activity" is intended polypeptides exhibiting activity similar, but not necessarily identical, to an activity of the AIM II protein of the invention, as measured in a particular biological assay. For example, AIM II protein cytotoxic activity can be measured using propidium iodide staining to demonstrate apoptosis as described by Zarres *et al., Cell 70*: 31-46 (1992).

Alternatively, AIMII induced apoptosis can also be measured using TUNEL staining as described by Gavierli *et al.*, *J. Cell. Biol. 119*: 493-501 (1992).

Briefly, the propidium iodide staining is performed as follows. Cells either from tissue or culture are fixed in formaldehyde, cut into frozen sections and stained with propidium iodide. The cell nuclei are visualized by propidium iodide using confocal fluorescent microscopy. Cell death is indicated by pyknotic nuclei (chromosome clumping, shrinking and/or fragmentation of nuclei).

Of course, due to the degeneracy of the genetic code, one of ordinary skill in the art will immediately recognize that a large number of the nucleic acid molecules having a sequence at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the nucleic acid sequence of the deposited cDNA or the nucleic acid sequence shown in Figures 1A-C (SEQ ID NO:1) will encode a polypeptide "having AIM II protein activity." In fact, since degenerate variants of these nucleotide sequences all encode the same polypeptide, this will be clear to the skilled artisan even without performing the above described comparison assay. It will be further recognized in the art that, for such nucleic acid molecules that are not degenerate variants, a reasonable number will also encode a polypeptide having AIM II protein activity. This is because the skilled artisan is fully aware of amino acid substitutions that are either less likely or not likely to significantly effect protein function (e.g., replacing one aliphatic amino acid with a second aliphatic amino acid).

For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie, J. U. *et al.*, "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions," *Science 247*:1306-1310 (1990), wherein the authors indicate that proteins are surprisingly tolerant of amino acid substitutions.

Vectors and Host Cells

The present invention also relates to vectors which include the isolated DNA molecules of the present invention, host cells which are genetically engineered with the recombinant vectors, and the production of AIM II polypeptides or fragments thereof by recombinant techniques.

The polynucleotides may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it may be packaged *in vitro* using an appropriate packaging cell line and then transduced into host cells.

The DNA insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the *E. coli lac, trp* and *tac* promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination and, in the transcribed region, a ribosome binding site for translation. The coding portion of the mature transcripts expressed by the constructs will preferably include a translation initiating at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase or neomycin resistance for eukaryotic cell culture and tetracycline or ampicillin resistance genes for culturing in *E. coli* and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as *E. coli*, *Streptomyces* and *Salmonella typhimurium* cells; fungal cells, such as yeast cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-9, available from Qiagen; pBS vectors, Phagescript vectors, Bluescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Other suitable vectors will be readily apparent to the skilled artisan.

Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection or other methods. Such methods are described in many standard laboratory manuals, such as Davis *et al., Basic Methods In Molecular Biology* (1986).

The polypeptide may be expressed in a modified form, such as a fusion protein, and may include not only secretion signals, but also additional heterologous functional regions. For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence in the host cell, during purification, or during subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to polypeptides to engender secretion or excretion, to improve stability and to facilitate purification, among others, are familiar and routine techniques in the art. A preferred fusion protein comprises a heterologous region from immunoglobulin that is useful to solubilize proteins. For example, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is thoroughly advantageous for use in therapy and diagnosis and thus results, for example, in improved pharmacokinetic properties (EP-A 0232 262). On the other

hand, for some uses it would be desirable to be able to delete the Fc part after the fusion protein has been expressed, detected and purified in the advantageous manner described. This is the case when Fc portion proves to be a hindrance to use in therapy and diagnosis, for example when the fusion protein is to be used as antigen for immunizations. In drug discovery, for example, human proteins, such as, hIL5- has been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. See, D. Bennett *et al.*, *Journal of Molecular Recognition*, Vol. 8:52-58 (1995) and K. Johanson *et al.*, *The Journal of Biological Chemistry*, Vol. 270, No. 16:9459-9471 (1995).

The AIM II protein can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification. Polypeptides of the present invention include naturally purified products, products of chemical synthetic procedures, and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes.

AIM II Polypeptides and Fragments

The invention further provides an isolated AIM II polypeptide having the amino acid sequence encoded by the deposited cDNA, or the amino acid sequence in Figures 1A-C (SEQ ID NO:2), or a peptide or polypeptide comprising a protion of the above polypeptides.

It will be recognized in the art that some amino acid sequences of the AIM II polypeptide can be varied without significant effect of the structure or function of the protein. If such differences in sequence are contemplated, it should be remembered that there will be critical areas on the protein which determine activity.

Thus, the invention further includes variations of the AIM II polypeptide which show substantial AIM II polypeptide activity or which include regions of AIM II protein such as the protein portions discussed below. Such mutants include deletions, insertions, inversions, repeats, and type substitutions. As indicated above, guidance concerning which amino acid changes are likely to be phenotypically silent can be found in Bowie, J.U., *et al.*, "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions," *Science 247:*1306-1310 (1990).

Thus, the fragment, derivative or analog of the polypeptide of Figures 1A-C (SEQ ID NO:2), or that encoded by the deposited cDNA, may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the mature polypeptide, such as an IgG Fc fusion region peptide or leader or secretory sequence or a sequence which is employed for purification of the mature polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

Of particular interest are substitutions of charged amino acids with another charged amino acid and with neutral or negatively charged amino acids. The latter results in proteins with reduced positive charge to improve the

characteristics of the AIM II protein. The prevention of aggregation is highly desirable. Aggregation of proteins not only results in a loss of activity but can also be problematic when preparing pharmaceutical formulations, because they can be immunogenic. (Pinckard *et al., Clin Exp. Immunol. 2*:331-340 (1967); Robbins *et al., Diabetes 36*:838-845 (1987); Cleland *et al. Crit. Rev. Therapeutic Drug Carrier Systems 10*:307-377 (1993)).

The replacement of amino acids can also change the selectivity of binding to cell surface receptors. Ostade *et al., Nature 361*:266-268 (1993) describes certain mutations resulting in selective binding of TNF-α to only one of the two known types of TNF receptors. Thus, the AIM II receptor of the present invention may include one or more amino acid substitutions, deletions or additions, either from natural mutations or human manipulation.

As indicated, changes are preferably of a minor nature, such as conservative amino acid substitutions that do not significantly affect the folding or activity of the protein (see Table 1).

TABLE 1. Conservative Amino Acid Substitutions.

Aromatic	Phenylalanine Tryptophan Tyrosine
Hydrophobic	Leucine Isoleucine Valine
Polar	Glutamine Asparagine
Basic	Arginine Lysine Histidine
Acidic	Aspartic Acid Glutamic Acid
Small	Alanine Serine Threonine Methionine Glycine

Amino acids in the AIM II protein of the present invention that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, *Science 244*:1081-1085 (1989)). The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for biological activity such as receptor binding or *in vitro*, or *in vitro* proliferative activity. Sites that are critical for ligand-receptor binding can also be determined by structural analysis such as crystallization, nuclear magnetic resonance or photoaffinity labeling (Smith *et al., J. Mol. Biol. 224*:899-904 (1992) and de Vos *et al. Science 255*:306-312 (1992)).

The polypeptides of the present invention are preferably provided in an isolated form. By "isolated polypeptide" is intended a poypeptide removed from its native environment. Thus, a polypeptide produced and contained within a recombinant host cell is considered "isolated" for purposes of the present invention. Also intended as an "isolated polypeptide" are polypeptides that have been purified, partially or substantially, from a recombinant host. For example, a recombinantly produced version of the AIM II polypeptide can be substantially purified by the one-step method described in Smith and Johnson, *Gene 67*:31-40 (1988).

The polypeptides of the present invention include the polypeptide encoded by the deposited cDNA, the polypeptide of Figures 1A-C (SEQ ID NO:2), the polypeptide of Figures 1A-C (SEQ ID NO:2) lacking the N-terminal methinone, the extracellular domain, the transmembrane domain, the intracellular domain, soluble polypeptides comprising all or part of the extracellular and intracelluar domains but lacking the transmembrane domain, as well as polypeptides which are at least 80% identical, more preferably at least 90% or 95% identical, still more preferably at least 96%, 97%, 98% or 99% identical to the polypeptide encoded by the deposited cDNA, to the polypeptide of Figures 1A-C (SEQ ID NO:2), and also include portions of such polypeptides with at least 30 amino acids and more preferably at least 50 amino acids.

By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a reference amino acid sequence of an AIM II polypeptide is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid of the AIM II polypeptide. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular polypeptide is at least 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the amino acid sequence shown in Figures 1A-C (SEQ ID NO:2) or to the amino acid sequence encoded by deposited cDNA clone can be determined conventionally using known computer programs such the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711. When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference amino acid sequence and that gaps in homology of up to 5% of the total number of amino acid residues in the reference sequence are allowed.

As used herein the term "AIM II" polypeptide includes membrane-bound proteins (comprising a cytoplasmic domain, a transmembrane domain, and an

extracellular domain) as well as truncated proteins that retain the AIM II polypeptide activity. In one embodiment, soluble AIM II polypeptides comprise all or part of the extracellular domain of an AIM II protein, but lack the transmsmbrane region that would cause retention of the polypeptide on a cell membrane. Soluble AIM II may also include part of the transmembrane region or part of the cytoplasmic domain or other sequences, provided that the soluble AIM II protein is capable of being secreted. A heterologous signal peptide can be fused to the N-terminus of the soluble AIM II polypeptide such that the soluble AIM II polypeptide is secreted upon expression.

The polypeptide of the present invention could be used as a molecular weight marker on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art.

In another aspect, the invention provides a peptide or polypeptide comprising an epitope-bearing portion of a polypeptide of the invention. The epitope of this polypeptide portion is an immunogenic or antigenic epitope of a polypeptide described herein. An "immunogenic epitope" is defined as a part of a protein that elicits an antibody response when the whole protein is the immunogen. On the other hand, a region of a protein molecule to which an antibody can bind is defined as an "antigenic epitope." The number of immunogenic epitopes of a protein generally is less than the number of antigenic epitopes. See, for instance, Geysen *et al., Proc. Natl. Acad. Sci. USA 81*:3998-4002 (1983).

As to the selection of peptides or polypeptides bearing an antigenic epitope (i.e., that contain a region of a protein molecule to which an antibody can bind), it is well known in that art that relatively short synthetic peptides that mimic part of a protein sequence are routinely capable of eliciting an antiserum that reacts with the partially mimicked protein. See, for instance, Sutcliffe, J. G., Shinnick, T. M., Green, N. and Learner, R.A. (1983) Antibodies that react with predetermined sites on proteins. *Science 219*:660-666. Peptides capable of eliciting protein-reactive sera are frequently represented in the primary sequence

of a protein, can be characterized by a set of simple chemical rules, and are confined neither to immunodominant regions of intact proteins (i.e., immunogenic epitopes) nor to the amino or carboxyl terminals.

Antigenic epitope-bearing peptides and polypeptides of the invention are therefore useful to raise antibodies, including monoclonal antibodies, that bind specifically to a polypeptide of the invention. See, for instance, Wilson *et al. Cell 37:* 767-778 (1984) at 777.

Antigenic epitope-bearing peptides and polypeptides of the invention preferably contain a sequence of at least seven, more preferably at least nine and most preferably between about at least about 15 to about 30 amino acids contained within the amino acid sequence of a polypeptide of the invention.

Non-limiting examples of antigenic polypeptides or peptides that can be used to generate AIM II-specific antibodies include: a polypeptide comprising amino acid residues from about 13 to about 20 in Figures 1A-C (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 23 to about 36 in Figures 1A-C (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 69 to about 79 in Figures 1A-C (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 85 to about 94 in Figures 1A-C (SEQ ID NO:2);a polypeptide comprising amino acid residues from about 167 to about 178 in Figures 1A-C (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 184 to about 196 in Figures 1A-C (SEQ ID NO:2); and a polypeptide comprising amino acid residues from about 221 to about 233 in Figures 1A-C (SEQ ID NO:2). As indicated above, the inventors have determined that the above polypeptide fragments are antigenic regions of the AIM II protein.

The epitope-bearing peptides and polypeptides of the invention may be produced by any conventional means. Houghten, R. A. (1985) General method for the rapid solid-phase synthesis of large numbers of peptides: specificity of antigen-antibody interaction at the level of individual amino acids. *Proc. Natl. Acad. Sci. USA 82*:5131-5135. This "Simultaneous Multiple Peptide Synthesis

(SMPS)" process is further described in U.S. Patent No. 4,631,211 to Houghten *et al.* (1986).

The AIM II polypeptide of the present invention may be employed to treat lymphoproliferative disease which results in lymphadenopathy, the AIM II mediates apoptosis by stimulating clonal deletion of T-cells and may therefore, be employed to treat autoimmune disease, to stimulate peripheral tolerance and cytotoxic T-cell mediated apoptosis. The AIM II may also be employed as a research tool in elucidating the biology of autoimmune disorders including systemic lupus erythematosus (SLE), immunoproliferative disease lymphadenopathy (IPL), angioimmunoproliferative lymphadenopathy (AIL), immunoblastive lymphadenopathy (IBL), rheumatoid arthritis, diabetes, and multiple sclerosis, allergies and to treat graft versus host disease.

The AIM II polypeptide of the present invention may also be employed to inhibit neoplasia, such as tumor cell growth. The AIM II polypeptide may be responsible for tumor destruction through apoptosis and cytotoxicity to certain cells. AIM II may also be employed to treat diseases which require growth promotion activity, for example, restenosis, since AIM II has proliferation effects on cells of endothelial origin. AIM II may, therefore, also be employed to regulate hematopoiesis in endothelial cell development.

This invention also provides a method for identification of molecules, such as receptor molecules, that bind AIM II. Genes encoding proteins that bind AIM II, such as receptor proteins, can be identified by numerous methods known to those of skill in the art, for example, ligand panning and FACS sorting. Such methods are described in many laboratory manuals such as, for instance, Coligan *et al.*, *Current Protocols in Immunology, 1(2)*:Chapter 5 (1991).

For instance, expression cloning may be employed for this purpose. To this end polyadenyiated RNA is prepared from a cell responsive to AIM II, a cDNA library is created from this RNA, the library is divided into pools and the pools are transfected individually into cells that are not responsive to AIM II. The transfected cells then are exposed to labeled AIM II. (AIM II can be labeled

by a variety of well-known techniques including standard methods of radio-iodination or inclusion of a recognition site for a site-specific protein kinase.) Following exposure, the cells are fixed and binding of AIM II is determined. These procedures conveniently are carried out on glass slides.

Pools are identified of cDNA that produced AIM II-binding cells. Sub-pools are prepared from these positives, transfected into host cells and screened as described above. Using an iterative sub-pooling and re-screening process, one or more single clones that encode the putative binding molecule, such as a receptor molecule, can be isolated.

Alternatively a labeled ligand can be photo affinity linked to a cell extract, such as a membrane or a membrane extract, prepared from cells that express a molecule that it binds, such as a receptor molecule. Cross-linked material is resolved by polyacrylamide gel electrophoresis ("PAGE") and exposed to X-ray film. The labeled complex containing the ligand-receptor can be excised, resolved into peptide fragments, and subjected to protein microsequencing. The amino acid sequence obtained from microsequencing can be used to design unique or degenerate oligonucleotide probes to screen cDNA libraries to identify genes encoding the putative receptor molecule.

Polypeptides of the invention also can be used to assess AIM II binding capacity of AIM II binding molecules, such as receptor molecules, in cells or in cell-free preparations.

As one of skill in the art will appreciate, AIM II polypeptides of the present invention and the epitope-bearing fragments thereof described above can be combined with parts of the constant domain of immunoglobulins (IgG), resulting in chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life *in vivo*. This has been shown, e.g., for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins (EPA 394,827; Traunecker *et al.*, *Nature 331*:84-86 (1988)). Fusion proteins that have a disulfide-linked dimeric structure due to

the IgG part can also be more efficient in binding and neutralizing other molecules than the monomeric AIM II protein or protein fragment alone (Fountoulakis *et al., J Biochem 270*:3958-3964 (1995)).

The present inventors have discovered that AIM II is expressed in spleen, thymus and bone marrow tissue. For a number of disorders, such as septic shock, inflammation, cerebral malaria, activation of the HIV virus, graft-host rejection, bone resorption, rheumatoid arthritis and cachexia, it is believed that significantly higher or lower levels of AIM II gene expression can be detected in certain tissues (e.g., spleen, thymus and bone marrow tissue) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to a "standard" AIM II gene expression level, i.e., the AIM II expression level in tissue or bodily fluids from an individual not having the disorder. Thus, the invention provides a diagnostic method useful during diagnosis of a disorder, which involves: (a) assaying AIM II gene expression level in cells or body fluid of an individual; (b) comparing the AIM II gene expression level with a standard AIM II gene expression level, whereby an increase or decrease in the assayed AIM II gene expression level compared to the standard expression level is indicative of a disorder.

AIM II Agonists and Antagonists

The invention also provides a method of screening compounds to identify those which enhance or block the action of AIM II on cells, such as its interaction with AIM II-binding molecules such as receptor molecules. An agonist is a compound which increases the natural biological functions of AIM II or which functions in a manner similar to AIM II. while antagonists decrease or eliminate such functions.

For example, a cellular compartment, such as a membrane preparation, may be prepared from a cell that expresses a molecule that binds AIM II, such as a molecule of a signaling or regulatory pathway modulated by AIM II. The

preparation is incubated with labeled AIM II in the absence or the presence of a candidate molecule which may be an AIM II agonist or antagonist. The ability of the candidate molecule to bind the binding molecule or AIM II itself is reflected in decreased binding of the labeled ligand. Molecules which bind gratuitously, *i.e.*, without inducing the effects of AIM II when bound to the AIM II binding molecule, are most likely to be good antagonists. Molecules that bind well and elicit effects that are the same as or closely related to AIM II, are good agonists.

AIM II-like effects of potential agonists and antagonists may by measured, for instance, by determining activity of a second messenger system following interaction of the candidate molecule with a cell or appropriate cell preparation, and comparing the effect with that of AIM II or molecules that elicit the same effects as AIM II. Second messenger systems that may be useful in this regard include but are not limited to AMP guanylate cyclase, ion channel or phosphoinositide hydrolysis second messenger systems.

Another example of an assay for AIM II antagonists is a competitive assay that combines AIM II and a potential antagonist with membrane-bound AIM II receptor molecules or recombinant AIM II receptor molecules under appropriate conditions for a competitive inhibition assay. AIM II can be labeled, such as by radioactivity, such that the number of AIM II molecules bound to a receptor molecule can be determined accurately to assess the effectiveness of the potential antagonist.

Potential antagonists include small organic molecules, peptides, polypeptides and antibodies that bind to a polypeptide of the invention, and thereby inhibit or extinguish its activity. Potential antagonists also may be small organic molecules, a peptide, a polypeptide such as a closely related protein or antibody that binds the same sites on a binding molecule, such as a receptor molecule, without inducing AIM II-induced activities, thereby preventing the action of AIM II by excluding AIM II from binding.

Other potential antagonists include antisense molecules. Antisense technology can be used to control gene expression through antisense DNA or RNA or through triple-helix formation. Antisense techniques are discussed, for example, in Okano, J. *Neurochem. 56*:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance, Lee *et al., Nucleic Acids Research* 6:3073 (1979); Cooney *et al., Science 241*:456 (1988); and Dervan *et al., Science 251*:1360 (1991). The methods are based on binding of a polynucleotide to a complementary DNA or RNA. For example, the 5' coding portion of a polynucleotide that encodes the mature polypeptide of the present invention may be used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription thereby preventing transcription and the production of AIM II. The antisense RNA oligonucleotide hybridizes to the mRNA *in vivo* and blocks translation of the mRNA molecule into AIM II polypeptide. The oligonucleotides described above can also be delivered to cells such that the antisense RNA or DNA may be expressed *in vivo* to inhibit production of AIM II.

The antagonists may be employed in a composition with a pharmaceutically acceptable carrier, e.g., as hereinafter described.

The antagonists may be employed for instance to treat cachexia which is a lipid clearing defect resulting from a systemic deficiency of lipoprotein lipase, which is believed to be suppressed by AIM II. The AIM II antagonists may also be employed to treat cerebral malaria in which AIM II may play a pathogenic role. The antagonists may also be employed to treat rheumatoid arthritis by inhibiting AIM-II induced production of inflammatory cytokines, such as IL1 in the synovial cells. When treating arthritis, AIM II antagonists are preferably injected intra-articularly.

The AIM II antagonists may also be employed to prevent graft-host rejection by preventing the stimulation of the immune system in the presence of a graft.

The AIM II antagonists may also be employed to inhibit bone resorption and, therefore, to treat and/or prevent osteoporosis.

The antagonists may also be employed as anti-inflammatory agents, and to treat endotoxic shock. This critical condition results from an exaggerated response to bacterial and other types of infection.

Cancer Prognosis

It is believed that certain tissues in mammals with cancer express significantly reduced levels of the AIM II protein and mRNA encoding the AIM II protein when compared to a corresponding "standard" mammal, i.e., a mammal of the same species not having the cancer. Further, it is believed that reduced levels of the AIM II protein can be detected in certain body fluids (e.g., sera, plasma, urine, and spinal fluid) from mammals with cancer when compared to sera from mammals of the same species not having the cancer. Thus, the invention provides a diagnostic method useful during tumor diagnosis, which involves assaying the expression level of the gene encoding the AIM II protein in mammalian cells or body fluid and comparing the gene expression level with a standard AIM II gene expression level, whereby an decrease in the gene expression level over the standard is indicative of certain tumors.

Where a tumor diagnosis has already been made according to conventional methods, the present invention is useful as a prognostic indicator, whereby patients exhibiting enhanced AIM II gene expression may experience a better clinical outcome relative to patients expressing the gene at a lower level.

By "assaying the expression level of the gene encoding the AIM II protein" is intended qualitatively or quantitatively measuring or estimating the level of the AIM II protein or the level of the mRNA encoding the AIM II protein in a first biological sample either directly (e.g., by determining or

estimating absolute protein level or mRNA level) or relatively (e.g., by comparing to the AIM II protein level or mRNA level in a second biological sample).

Preferably, the AIM II protein level or mRNA level in the first biological sample is measured or estimated and compared to a standard AIM II protein level or mRNA level, the standard being taken from a second biological sample obtained from an individual not having the cancer. As will be appreciated in the art, once a standard AIM II protein level or mRNA level is known, it can be used repeatedly as a standard for comparison.

By "biological sample" is intended any biological sample obtained from an individual, cell line, tissue culture, or other source which contains AIM II protein or mRNA. Biological samples include mammalian body fluids (such as sera, plasma, urine, synovial fluid and spinal fluid) which contain secreted mature AIM II protein, and ovarian, prostate, heart, placenta, pancreas liver, spleen, lung, breast and umbilical tissue.

The present invention is useful for detecting cancer in mammals. In particular the invention is useful during diagnosis of the of following types of cancers in mammals: breast, ovarian, prostate, bone, liver, lung, pancreatic, and spleenic. Preferred mammals include monkeys, apes, cats, dogs, cows, pigs, horses, rabbits and humans. Particularly preferred are humans.

Total cellular RNA can be isolated from a biological sample using the single-step guanidinium-thiocyanate-phenol-chloroform method described in Chomczynski and Sacchi, *Anal. Biochem. 162:*156-159 (1987). Levels of mRNA encoding the AIM II protein are then assayed using any appropriate method. These include Northern blot analysis (Harada *et al., Cell 63*:303-312 (1990)), S1 nuclease mapping (Fujita *et al., Cell 49:*357-367 (1987)) , the polymerase chain reaction (PCR), reverse transcription in combination with the polymerase chain reaction (RT-PCR) (Makino *et al., Technique 2*:295-301 (1990)) , and reverse transcription in combination with the ligase chain reaction (RT-LCR).

Assaying AIM II protein levels in a biological sample can occur using antibody-based techniques. For example, AIM II protein expression in tissues can be studied with classical immunohistological methods (Jalkanen, M., *et al.*, *J. Cell. Biol.* *101*:976-985 (1985); Jalkanen, M., *et al.*, *J. Cell . Biol.* *105*:3087-3096 (1987)).

Other antibody-based methods useful for detecting AIM II protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA).

Suitable lables are known in the art and include enzyme lables, such as, Glucose oxidase, and radioisotopes, such as iodine (^{125}I, ^{121}I), carbon (^{14}C), sulpher (^{35}S), tritium (^{3}H), indium (^{112}In), and technetium (^{99m}Tc), and fluorescent labels, such as fluorescein and rhodamine, and biotin.

Therapeutics

The AIM II polypeptides, particularly human AIM-II polypeptides, may be employed to treat neoplasia, lymphadenopathy, autoimmune disease, graft versus host disease. In addition, the AIM II polypeptide of the present invention may be employed to inhibit neoplasia, such as tumor cell growth. The AIM II polypeptide may be responsible for tumor destruction through apoptosis and cytotoxicity to certain cells. AIM II may also be employed to treat diseases which require growth promotion activity, for example, restenosis, since AIM II has proliferation effects on cells of endothelial origin. AIM II may, therefore, also be employed to regulate hematopoiesis in endothelial cell development.

Modes of administration

It will be appreciated that conditions, such as those discussed above, can be treated by administration of AIM II protein. Thus, the invention further provides a method of treating an individual in need of an increased level of AIM II activity comprising administering to such an individual a pharmaceutical

composition comprising an effective amount of an isolated AIM II polypeptide of the invention, particularly a mature form of the AIM II, effective to increase the AIM II activity level in such an individual.

As a general proposition, the total pharmaceutically effective amount of AIM II polypeptide administered parenterally per dose will be in the range of about 1 μg/kg/day to 10 mg/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01 mg/kg/day, and most preferably for humans between about 0.01 and 1 mg/kg/day for the hormone. If given continuously, the AIM II polypeptide is typically administered at a dose rate of about 1 μg/kg/hour to about 50 μg/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed.

Pharmaceutical compositions containing the AIM II of the invention may be administered orally, rectally, parenterally, intracistemally, intravaginally, intraperitoneally, topically (as by powders, ointments, drops or transdermal patch), bucally, or as an oral or nasal spray. By "pharmaceutically acceptable carrier" is meant a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

In addition to soluble AIM II polypeptides, AIM II polypeptides containing the transmembrane region can also be used when appropriately solubilized by including detergents, such as triton X-100, with buffer.

Chromosome Assays

The nucleic acid molecules of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can

hybridize with a particular location on an individual human chromosome. The mapping of DNAs to chromosomes according to the present invention is an important first step in correlating those sequences with genes associated with disease.

In certain preferred embodiments in this regard, the cDNA herein disclosed is used to clone genomic DNA of an AIM II protein gene. This can be accomplished using a variety of well known techniques and libraries, which generally are available commercially. The genomic DNA then is used for *in situ* chromosome mapping using well known techniques for this purpose.

In addition, in some cases, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the cDNA. Computer analysis of the 3′ untranslated region of the gene is used to rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes.

Fluorescence *in situ* hybridization ("FISH") of a cDNA clone to a metaphase chromosomal spread can be used to provide a precise chromosomal location in one step. This technique can be used with probes from the cDNA as short as 50 or 60 bp. For a review of this technique, see Verma *et al., Human Chromosomes: A Manual Of Basic Techniques*, Pergamon Press, New York (1988).

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, *Mendelian Inheritance In Man*, available on-line through Johns Hopkins University, Welch Medical Library. The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).

Next, it is necessary to determine the differences in the cDNA or genomic sequence between affected and unaffected individuals. If a mutation is observed

in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

Examples

Example 1: Expression and Purification of AIM II in E. coli

A. Expression of AIM II with an N-terminal HA tag.

The DNA sequence encoding the AIM II protein in the deposited cDNA clone is amplified using PCR oligonucleotide primers specific to the amino terminal sequences of the AIM II protein and to vector sequences 3′ to the gene. Additional nucleotides containing restriction sites to facilitate cloning are added to the 5′ and 3′ sequences respectively.

A 22 kDa AIM II protein fragment (lacking the N-terminus and transmembrane region) is expressed using the following primers:

The 5′ oligonucleotide primer has the sequence 5′ GCGGGATCCGGAGAGATGGTCACC 3′ (SEQ ID NO:7) containing the underlined BamHI restriction site, which encodes 244-258 nucleotides of the AIM II protein coding sequence in Figures 1A-C (SEQ ID NO:1).

The 3′ primer has the sequence 5′ CGCAAGCTTCCTTCACACCATGAAAGC 3′ (SEQ ID NO:8) containing the underlined Hind III restriction site followed by 756-783 nucleotides as shown in Figures 1A-C.

The entire AIM II protein can be expressed using the following primers:

The 5′ oligonucleotide primer has the sequence 5′ GACC GGATCC ATG GAG GAG AGT GTC GTA CGG C 3′ (SEQ ID NO:9) containing the underlined Bam HI restriction site, which encodes 22 nucleotides of the AIM II protein coding sequence in Figures 1A-C (SEQ ID NO:1).

The 3′ primer has the sequence 5′ CGC AAGCTT CCT TCA CAC CAT GAA AGC 3′ (SEQ ID NO:10) containing the underlined HindIII restriction site followed followed by 756-783 nucleotides as shown in Figures 1A-C.

The restriction sites are convenient to restriction enzyme sites in the bacterial expression vector pQE9, which are used for bacterial expression in these examples. (Qiagen, Inc. 9259 Eton Avenue, Chatsworth, CA, 91311). pQE9 encodes ampicillin antibiotic resistance ("Amp^r") and contains a bacterial origin of replication ("ori"), an IPTG inducible promoter, a ribosome binding site ("RBS"), a 6-His tag and restriction enzyme sites.

The amplified AIM II DNA and the vector pQE9 both are digested with BamHI and Hind III and the digested DNAs are then ligated together. Insertion of the AIM II protein DNA into the restricted pQE9 vector places the AIM II protein coding region downstream of and operably linked to the vector's IPTG-inducible promoter and in-frame with an initiating AUG appropriately positioned for translation of AIM II protein.

B. Expression of AIM II with a C-terminal HA tag

The bacterial expression vector pQE60 is used for bacterial expression in this example. (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311). pQE60 encodes ampicillin antibiotic resistance ("Amp^r") and contains a bacterial origin of replication ("ori"), an IPTG inducible promoter, a ribosome binding site ("RBS"), six codons encoding histidine residues that allow affinity purification using nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin sold by QIAGEN, Inc., *supra*, and suitable single restriction enzyme cleavage sites. These elements are arranged such that an inserted DNA fragment encoding a polypeptide expresses that polypeptide with the six His residues (i.e., a "6 X His tag") covalently linked to the carboxyl terminus of that polypeptide.

The DNA sequence encoding the desired portion of the AIM II protein is amplified from the deposited cDNA clone using PCR oligonucleotide primers which anneal to the amino terminal sequences of the desired portion of the AIM II protein and to sequences in the deposited construct 3' to the cDNA coding

sequence. Additional nucleotides containing restriction sites to facilitate cloning in the pQE60 vector are added to the 5' and 3' sequences, respectively.

For cloning the protein, the 5' primer has the sequence 5' GACGC CCATGG AG GAG GAG AGT GTC GTA CGG C 3' (SEQ ID NO: 17) containing the underlined NcoI restriction site followed by nucleotides complementary to the amino terminal coding sequence of the AIM II sequence in Figures 1A-C. One of ordinary skill in the art would appreciate, of course, that the point in the protein coding sequence where the 5' primer begins may be varied to amplify a DNA segment encoding any desired portion of the complete protein (shorter or longer). The 3' primer has the sequence 5' GACC GGATCC CAC CAT GAA AGC CCC GAA GTA AG 3' (SEQ ID NO: 18) containing the underlined BamHI restriction site followed by nucleotides complementary to the 3' end of the coding sequence immediately before the stop codon in the AIM II DNA sequence in Figures 1A-C, with the coding sequence aligned with the restriction site so as to maintain its reading frame with that of the six His codons in the pQE60 vector.

The amplified AIM II DNA fragment and the vector pQE60 are digested with BamHI and Nco I and the digested DNAs are then ligated together. Insertion of the AIM II DNA into the restricted pQE60 vector places the AIM II protein coding region downstream from the IPTG-inducible promoter and in-frame with an initiating AUG and the six histidine codons.

The ligation mixture from the HA tagged expression constructs made in A or B, above, is transformed into competent *E. coli* cells using standard procedures. Such procedures are described in Sambrook *et al.*, Molecular Cloning: a Laboratory Manual, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989). *E. coli* strain M15/rep4, containing multiple copies of the plasmid pREP4, which expresses lac repressor and confers kanamycin resistance ("Kan^r"), is used in carrying out the illustrative example described herein. This strain, which is only one of many that are suitable for expressing AIM II protein, is available commercially from Qiagen.

Transformants are identified by their ability to grow on LB plates in the presence of ampicillin and kanamycin. Plasmid DNA is isolated from resistant colonies and the identity of the cloned DNA confirmed by restriction analysis.

Clones containing the desired constructs are grown overnight ("O/N") in liquid culture in LB media supplemented with both ampicillin (100 μg/ml) and kanamycin (25 μg/ml).

The O/N culture is used to inoculate a large culture, at a dilution of approximately 1:100 to 1:250. The cells are grown to an optical density at 600nm ("OD600") of between 0.4 and 0.6. Isopropyl-B-D-thiogalactopyranoside ("IPTG") is then added to a final concentration of 1 mM to induce transcription from *lac* repressor sensitive promoters, by inactivating the *lac*I repressor. Cells subsequently are incubated further for 3 to 4 hours. Cells then are harvested by centrifugation and disrupted, by standard methods. Inclusion bodies are purified from the disrupted cells using routine collection techniques, and protein is solubilized from the inclusion bodies into 8M urea. The 8M urea solution containing the solubilized protein is passed over a PD-10 column in 2X phosphate-buffered saline ("PBS"), thereby removing the urea, exchanging the buffer and refolding the protein. The protein is purified by a further step of chromatography to remove endotoxin. Then, it is sterile filtered. The sterile filtered protein preparation is stored in 2X PBS at a concentration of 95 μ/ml.

C. Expression and Purification of AIM II without an HA tag

The bacterial expression vector pQE60 is used for bacterial expression in this example. (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311). pQE60 encodes ampicillin antibiotic resistance ("Amp^r") and contains a bacterial origin of replication ("ori"), an IPTG inducible promoter, a ribosome binding site ("RBS"), six codons encoding histidine residues that allow affinity purification using nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin sold by QIAGEN, Inc., *supra,* and suitable single restriction enzyme cleavage sites. These elements are arranged such that a DNA fragment encoding a polypeptide may be inserted

in such as way as to produce that polypeptide with the six His residues (i.e., a "6 X His tag") covalently linked to the carboxyl terminus of that polypeptide. However, in this example, the polypeptide coding sequence is inserted such that translation of the six His codons is prevented and, therefore, the polypeptide is produced with no 6 X His tag.

The DNA sequence encoding the desired portion of the AIM II protein lacking the hydrophobic leader sequence is amplified from the deposited cDNA clone using PCR oligonucleotide primers which anneal to the amino terminal sequences of the desired portion of the AIM II protein and to sequences in the deposited construct 3' to the cDNA coding sequence. Additional nucleotides containing restriction sites to facilitate cloning in the pQE60 vector are added to the 5' and 3' sequences, respectively.

For cloning the protein, the 5' primer has the sequence 5'GACGC <u>CCATGG</u> AG GAG GAG AGT GTC GTA CGG C 3' (SEQ ID NO: 17) containing the underlined NcoI restriction site followed by nucleotides complementary to the amino terminal coding sequence of the AIM II sequence in Figures 1A-C. One of ordinary skill in the art would appreciate, of course, that the point in the protein coding sequence where the 5' primer begins may be varied to amplify a desired portion of the complete protein (i.e., shorter or longer). The 3' primer has the sequence 5' CGC <u>AAGCTT</u> CCTT CAC ACC ATG AAA GC 3' (SEQ ID NO: 19) containing the underlined Hind III restriction site followed by nucleotides complementary to the 3' end of the non-coding sequence in the AIM II DNA sequence in Figures 1A-C.

The amplified AIM II DNA fragments and the vector pQE60 are digested with NcoI and Hind III and the digested DNAs are then ligated together. Insertion of the AIM II DNA into the restricted pQE60 vector places the AIM II protein coding region including its associated stop codon downstream from the IPTG-inducible promoter and in-frame with an initiating AUG. The associated stop codon prevents translation of the six histidine codons downstream of the insertion point.

The ligation mixture is transformed into competent *E. coli* cells using standard procedures such as those described in Sambrook *et al., Molecular Cloning: a Laboratory Manual, 2nd Ed.;* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989). *E. coli* strain M15/rep4, containing multiple copies of the plasmid pREP4, which expresses the lac repressor and confers kanamycin resistance ("Kan^r"), is used in carrying out the illustrative example described herein. This strain, which is only one of many that are suitable for expressing AIM II protein, is available commercially from QIAGEN, Inc., *supra.* Transformants are identified by their ability to grow on LB plates in the presence of ampicillin and kanamycin. Plasmid DNA is isolated from resistant colonies and the identity of the cloned DNA confirmed by restriction analysis, PCR and DNA sequencing.

Clones containing the desired constructs are grown overnight ("O/N") in liquid culture in LB media supplemented with both ampicillin (100 μg/ml) and kanamycin (25 μg/ml). The O/N culture is used to inoculate a large culture, at a dilution of approximately 1:25 to 1:250. The cells are grown to an optical density at 600 nm ("OD600") of between 0.4 and 0.6. Isopropyl-b-D-thiogalactopyranoside ("IPTG") is then added to a final concentration of 1 mM to induce transcription from the lac repressor sensitive promoter, by inactivating the lacI repressor. Cells subsequently are incubated further for 3 to 4 hours. Cells then are harvested by centrifugation.

The cells are then stirred for 3-4 hours at 4°C in 6M guanidine-HCl, pH8. The cell debris is removed by centrifugation, and the supernatant containing the AIM II is dialyzed against 50 mM Na-acetate buffer pH6, supplemented with 200 mM NaCl. Alternatively, the protein can be successfully refolded by dialyzing it against 500 mM NaCl, 20% glycerol, 25 mM Tris/HCl pH7.4, containing protease inhibitors. After renaturation the protein can be purified by ion exchange, hydrophobic interaction and size exclusion chromatography. Alternatively, an affinity chromatography step such as an antibody column can

be used to obtain pure AIM II protein. The purified protein is stored at 4°C or frozen at -80°C.

Example 2: Cloning and Expression of AIM II protein in a Baculovirus Expression System

The cDNA sequence encoding the full length AIM II protein in the deposited clone is amplified using PCR oligonucleotide primers corresponding to the 5′ and 3′ sequences of the gene:

The 5′ primer has the sequence 5′GCT CCA GGA TCC GCC ATC ATG GAG GAG AGT GTC GTA CGG C3′ (SEQ ID NO:11) containing the underlined Bam HI restriction enzyme site followed by 22 bases of the sequence of AIM II protein in Figures 1A-C. Inserted into an expression vector, as described below, the 5′ end of the amplified fragment encoding AIM II provides an efficient signal peptide. An efficient signal for initiation of translation in eukaryotic cells, as described by Kozak, M., *J. Mol. Biol. 196*:947-950 (1987) is appropriately located in the vector portion of the construct.

The 3′ primer has the sequence 5′GA CGC GGT ACC GTC CAA TGC ACC ACG CTC CTT CCT TC 3′ (SEQ ID NO:12) containing the underlined Asp 718 restriction site followed by nucleotides complementary to 769-795 nucleotides of the AIM II set out in Figures 1A-C.

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("Geneclean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with Bam HI and Asp 718 and again is purified on a 1% agarose gel. This fragment is designated herein F2.

The vector pA2-GP is used to express the AIM II protein in the baculovirus expression system, using standard methods, as described in Summers *et al.*, A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures, Texas Agricultural Experimental Station Bulletin No. 1555 (1987). This expression vector contains the strong polyhedrin promoter of the

Autographa californica nuclear polyhedrosis virus (AcMNPV) followed by convenient restriction sites. The signal peptide of AcMNPV gp67, including the N-terminal methionine, is located just upstream of a BamHI site. The polyadenylation site of the simian virus 40 ("SV40") is used for efficient polyadenylation. For an easy selection of recombinant virus the beta-galactosidase gene from *E. coli* is inserted in the same orientation as the polyhedrin promoter and is followed by the polyadenylation signal of the polyhedrin gene. The polyhedrin sequences are flanked at both sides by viral sequences for cell-mediated homologous recombination with wild-type viral DNA to generate viable virus that express the cloned polynucleotide.

Many other baculovirus vectors could be used in place of pA2-GP, such as pAc373, pVL941 and pAcIM1 provided, as those of skill readily will appreciate, that construction provides appropriately located signals for transcription, translation, trafficking and the like, such as an in-frame AUG and a signal peptide, as required. Such vectors are described in Luckow *et al.*, *Virology 170*: 31-39, among others.

The plasmid is digested with the restriction enzyme Bam HI and Asp 718 and then is dephosphorylated using calf intestinal phosphatase, using routine procedures known in the art. The DNA is then isolated from a 1% agarose gel using a commercially available kit ("Geneclean" BIO 101 Inc., La Jolla, Ca.). This vector DNA is designated herein "V".

Fragment F2 and the dephosphorylated plasmid V2 are ligated together with T4 DNA ligase. *E. coli* HB101 cells are transformed with ligation mix and spread on culture plates. Bacteria are identified that contain the plasmid with the human AIM II gene by digesting DNA from individual colonies using XbaI and then analyzing the digestion product by gel electrophoresis. The sequence of the cloned fragment is confirmed by DNA sequencing. This plasmid is designated herein pBac AIM II .

5 µg of the plasmid pBac AIM II is co-transfected with 1.0 µg of a commercially available linearized baculovirus DNA ("BaculoGold™ baculovirus

DNA", Pharmingen, San Diego, CA.), using the lipofection method described by Felgner *et al.*, Proc. Natl. Acad. Sci. USA 84: 7413-7417 (1987). 1µg of BaculoGold™ virus DNA and 5 µg of the plasmid pBac AIM II are mixed in a sterile well of a microtiter plate containing 50 µl of serum-free Grace's medium (Life Technologies Inc., Gaithersburg, MD). Afterwards 10 µl Lipofectin plus 90 µl Grace's medium are added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture is added drop-wise to Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml Grace's medium without serum. The plate is rocked back and forth to mix the newly added solution. The plate is then incubated for 5 hours at 27°C. After 5 hours the transfection solution is removed from the plate and 1 ml of Grace's insect medium supplemented with 10% fetal calf serum is added. The plate is put back into an incubator and cultivation is continued at 27°C for four days.

After four days the supernatant is collected and a plaque assay is performed, as described by Summers and Smith, cited above. An agarose gel with "Blue Gal" (Life Technologies Inc., Gaithersburg) is used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained plaques. (A detailed description of a "plaque assay" of this type can also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies Inc., Gaithersburg, page 9-10).

Four days after serial dilution, the virus is added to the cells. After appropriate incubation, blue stained plaques are picked with the tip of an Eppendorf pipette. The agar containing the recombinant viruses is then resuspended in an Eppendorf tube containing 200 µl of Grace's medium. The agar is removed by a brief centrifugation and the supernatant containing the recombinant baculovirus is used to infect Sf9 cells seeded in 35 mm dishes. Four days later the supernatants of these culture dishes are harvested and then they are stored at 4°C. A clone containing properly inserted hESSB I, II and III is identified by DNA analysis including restriction mapping and sequencing. This is designated herein as V-AIM II .

Sf9 cells are grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells are infected with the recombinant baculovirus V-AIM II at a multiplicity of infection ("MOI") of about 2 (about 1 to about 3). Six hours later the medium is removed and is replaced with SF900 II medium minus methionine and cysteine (available from Life Technologies Inc., Gaithersburg). 42 hours later, 5 μCi of ^{35}S-methionine and 5 μCi ^{35}S-cysteine (available from Amersham) are added. The cells are further incubated for 16 hours and then they are harvested by centrifugation, lysed and the labeled proteins are visualized by SDS-PAGE and autoradiography.

Example 3: Cloning and Expression in Mammalian Cells

Most of the vectors used for the transient expression of the AIM II protein gene sequence in mammalian cells should carry the SV40 origin of replication. This allows the replication of the vector to high copy numbers in cells (e.g., COS cells) which express the T antigen required for the initiation of viral DNA synthesis. Any other mammalian cell line can also be utilized for this purpose.

A typical mammalian expression vector contains the promoter element, which mediates the initiation of transcription of mRNA, the protein coding sequence, and signals required for the termination of trancription and polyadenylation of the transcript. Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription can be achieved with the early and late promoters from SV40, the long terminal repeats (LTRs) from Retroviruses, e.g., RSV, HTLVI, HIVI and the early promoter of the cytomegalovirus (CMV). However. cellular signals can also be used (e.g., human actin promoter). Suitable expression vectors for use in practicing the present invention include, for example. vectors such as pSVL and pMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146) and pBC12MI (ATCC 67109). Mammalian host cells that could be used include, human Hela, 283, H9

and Jurkart cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV1, African green monkey cells, quail QC1-3 cells, mouse L cells and Chinese hamster ovary cells.

Alternatively, the gene can be expressed in stable cell lines that contain the gene integrated into a chromosome. The co-transfection with a selectable marker such as dhfr, gpt, neomycin, hygromycin allows the identification and isolation of the transfected cells.

The transfected gene can also be amplified to express large amounts of the encoded protein. The DHFR (dihydrofolate reductase) is a useful marker to develop cell lines that carry several hundred or even several thousand copies of the gene of interest. Another useful selection marker is the enzyme glutamine synthase (GS) (Murphy *et al., Biochem J. 227:*277-279 (1991); Bebbington *et al., Bio/Technology 10:*169-175 (1992)). Using these markers, the mammalian cells are grown in selective medium and the cells with the highest resistance are selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) cells are often used for the production of proteins.

The expression vectors pC1 and pC4 contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen *et al., Molecular and Cellular Biology*, 438-447 (March, 1985)) plus a fragment of the CMV-enhancer (Boshart *et al., Cell 41*:521-530 (1985)). Multiple cloning sites, e.g., with the restriction enzyme cleavage sites BamHI, XbaI and Asp718, facilitate the cloning of the gene of interest. The vectors contain in addition the 3′ intron, the polyadenylation and termination signal of the rat preproinsulin gene.

Example 3(a): Cloning and Expression in COS Cells

The expression plasmid, pAIM II HA, is made by cloning a cDNA encoding AIM II into the expression vector pcDNAI/Amp (which can be obtained from Invitrogen, Inc.).

The expression vector pcDNAI/amp contains: (1) an *E.coli* origin of replication effective for propagation in *E. coli* and other prokaryotic cells; (2) an ampicillin resistance gene for selection of plasmid-containing prokaryotic cells; (3) an SV40 origin of replication for propagation in eukaryotic cells; (4) a CMV promoter, a polylinker, an SV40 intron, and a polyadenylation signal arranged so that a cDNA conveniently can be placed under expression control of the CMV promoter and operably linked to the SV40 intron and the polyadenylation signal by means of restriction sites in the polylinker.

A DNA fragment encoding the AIM II protein and an HA tag fused in frame to its 3′ end is cloned into the polylinker region of the vector so that recombinant protein expression is directed by the CMV promoter. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein described by Wilson *et al., Cell 37:* 767 (1984). The fusion of the HA tag to the target protein allows easy detection of the recombinant protein with an antibody that recognizes the HA epitope.

The plasmid construction strategy is as follows. The AIM II cDNA of the deposited clone is amplified using primers that contain convenient restriction sites, much as described above regarding the construction of expression vectors for expression of AIM II in *E. coli*. To facilitate detection, purification and characterization of the expressed AIM II, one of the primers contains a hemagglutinin tag ("HA tag") as described above.

Suitable primers include the following, which are used in this example. The 5′ primer, containing the underlined BamHI site, and an AUG start codon has the following sequence:

5′G CTC <u>GGA TCC</u> GCC ATC ATG 3′ (SEQ ID NO:13).

The 3′ primer, containing the underlined Xba I site, a stop codon, 9 codons thereafter forming the hemagglutinin HA tag, and 31 bp of 3′ coding sequence (at the 3′ end) has the following sequence:

5'GAT GTT CTA GAA AGC GTA GTC TGG GAC GTC GTA TGG GTA CAC CAT GAA AGC CCC GAA GTA AGA CCG GGT AC 3' (SEQ ID NO:14).

The PCR amplified DNA fragment and the vector, pcDNAI/Amp, are digested with HindIII and XhoI and then ligated. The ligation mixture is transformed into *E. coli* strain SURE (available from Stratagene Cloning Systems, 11099 North Torrey Pines Road, La Jolla, CA 92037), and the transformed culture is plated on ampicillin media plates which then are incubated to allow growth of ampicillin resistant colonies. Plasmid DNA is isolated from resistant colonies and examined by restriction analysis and gel sizing for the presence of the AIM II -encoding fragment.

For expression of recombinant AIM II, COS cells are transfected with an expression vector, as described above, using DEAE-DEXTRAN, as described, for instance, in Sambrook *et al.*, Molecular Cloning: a Laboratory Manual, Cold Spring Laboratory Press, Cold Spring Harbor, New York (1989). Cells are incubated under conditions for expression of AIM II by the vector.

Expression of the AIM II HA fusion protein is detected by radiolabelling and immunoprecipitation. using methods described in, for example Harlow *et al.*, Antibodies: A Laboratory Manual, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1988). To this end, two days after transfection, the cells are labeled by incubation in media containing ^{35}S-cysteine for 8 hours. The cells and the media are collected, and the cells are washed and the lysed with detergent-containing RIPA buffer: 150 mM NaCl, 1% NP-40, 0.1% SDS, 1% NP-40, 0.5% DOC, 50 mM TRIS, pH 7.5, as described by Wilson *et al.* cited above. Proteins are precipitated from the cell lysate and from the culture media using an HA-specific monoclonal antibody. The precipitated proteins then are analyzed by SDS-PAGE gels and autoradiography. An expression product of the expected size is seen in the cell lysate, which is not seen in negative controls.

Example 3(b): Cloning and Expression in CHO Cells

The vector pC4 is used for the expression of AIM II protein. Plasmid pC1 is a derivative of the plasmid pSV2-dhfr [ATCC Accession No. 37146]. Both plasmids contain the mouse DHFR gene under control of the SV40 early promoter. Chinese hamster ovary- or other cells lacking dihydrofolate activity that are transfected with these plasmids can be selected by growing the cells in a selective medium (alpha minus MEM, Life Technologies) supplemented with the chemotherapeutic agent methotrexate. The amplification of the DHFR genes in cells resistant to methotrexate (MTX) has been well documented (see, e.g., Alt, F.W., Kellems, R.M., Bertino, J.R., and Schimke, R.T., 1978, J. Biol. Chem. 253:1357-1370, Hamlin, J.L. and Ma, C. 1990, Biochem. et Biophys. Acta, 1097:107-143, Page, M.J. and Sydenham, M.A. 1991, Biotechnology Vol. 9:64-68). Cells grown in increasing concentrations of MTX develop resistance to the drug by overproducing the target enzyme, DHFR, as a result of amplification of the DHFR gene. If a second gene is linked to the DHFR gene it is usually co-amplified and over-expressed. It is state of the art to develop cell lines carrying more than 1,000 copies of the genes. Subsequently, when the methotrexate is withdrawn, cell lines contain the amplified gene integrated into the chromosome(s).

Plasmid pC4 contains for expressing the gene of interest the strong promoter of the long terminal repeat (LTR) of the Rous Sarcoma Virus (Cullen, *et al., Molecular and Cellular Biology,* March 1985:438-447) plus a fragment isolated from the enhancer of the immediate early gene of human cytomegalovirus (CMV) (Boshart *et al., Cell 41*:521-530 (1985)). Downstream of the promoter are Bam HI, XbaI, and Asp718 restriction enzyme cleavage sites that allow integration of the genes. Behind these cloning sites the plasmid contains the 3' intron and polyadenylation site of the rat preproinsulin gene. Other high efficiency promoters can also be used for the expression, e.g., the human β-actin promoter, the SV40 early or late promoters or the long terminal

repeats from other retroviruses, e.g., HIV and HTLVI. Clontech's Tet-Off and Tet-On gene expression systems and similar systems can be used to express the AIM II in a regulated way in mammalian cells (Gossen, M., & Bujard, H. 1992, *Proc. Natl. Acad. Sci. USA 89*: 5547-5551). For the polyadenylation of the mRNA other signals, e.g., from the human growth hormone or globin genes can be used as well. Stable cell lines carrying a gene of interest integrated into the chromosomes can also be selected upon co-transfection with a selectable marker such as gpt, G418 or hygromycin. It is advantageous to use more than one selectable marker in the beginning, e.g., G418 plus methotrexate.

The plasmid pC4 is digested with the restriction enzymes Bam HI and Asp 718 and then dephosphorylated using calf intestinal phosphatase by procedures known in the art. The vector is then isolated from a 1% agarose gel.

The DNA sequence encoding the complete AIM II protein including its leader sequence is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene. The 5' primer has the sequence 5'GCT CCA <u>GGA TCC</u> GCC ATC ATG GAG GAG AGT GTC GTA CGG C3' (SEQ ID NO:15) containing the underlined Bam HI restriction enzyme site followed by an efficient signal for initiation of translation in eukaryotes, as described by Kozak, M., *J. Mol. Biol. 196*:947-950 (1987), and 22 bases of the coding sequence of AIM II shown in Figures 1A-C (SEQ ID NO:1). The 3' primer has the sequence 5'GA CGC <u>GGT ACC</u> GTC CAA TGC ACC ACG CTC CTT CCT TC 3' (SEQ ID NO:16) containing the underlined Asp 718 restriction site followed by nucleotides complementary to nucleotides 769-795 of the AIM II gene shown in Figures 1A-C (SEQ ID NO:1).

The amplified fragment is digested with the endonucleases BamHI and Asp718 and then purified again on a 1% agarose gel. The isolated fragment and the dephosphorylated vector are then ligated with T4 DNA ligase. *E. coli* HB101 or XL-1 Blue cells are then transformed and bacteria are identified that contain the fragment inserted into plasmid pC4 using, for instance, restriction enzyme analysis.

Chinese hamster ovary cells lacking an active DHFR gene are used for transfection. 5 μg of the expression plasmid pC4 is cotransfected with 0.5 μg of the plasmid pSV2-neo using lipofectin (Felgner *et al., supra).* The plasmid pSV2neo contains a dominant selectable marker, the neo gene from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1 mg/ml G418. After 2 days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MEM supplemented with 10, 25, or 50 ng/ml of metothrexate plus 1 mg/ml G418. After about 10-14 days single clones are trypsinized and then seeded in 6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (1 μM, 2 μM, 5 μM, 10 mM, 20 mM). The same procedure is repeated until clones are obtained which grow at a concentration of 100 - 200 μM. Expression of the desired gene product is analyzed, for instance, by SDS-PAGE and Western blot or by reverse.phase HPLC analysis.

Example 4: Tissue distribution of AIM II protein expression

Northern blot analysis is carried out to examine AIM II gene expression in human tissues, using methods described by, among others, Sambrook *et al.*, cited above. A cDNA probe containing the entire nucleotide sequence of the AIM II protein (SEQ ID NO:1) is labeled with ^{32}P using the *redi*prime™ DNA labeling system (Amersham Life Science), according to manufacturer's instructions. After labelling, the probe is purified using a CHROMA SPIN-100™ column (Clontech Laboratories, Inc.), according to manufacturer's protocol number PT1200-1. The purified labelled probe is then used to examine various human tissues for AIM II mRNA.

Multiple Tissue Northern (MTN) blots containing various human tissues (H) or human immune system tissues (IM) are obtained from Clontech and are examined with labelled probe using ExpressHyb™ hybridization solution (Clontech) according to manufacturer's protocol number PT1190-1. Following hybridization and washing, the blots are mounted and exposed to film at -70°C overnight, and films developed according to standard procedures.

It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples.

Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

The entire disclosure of all publications (including patents, patent applications, journal articles, laboratory manuals, books, or other documents) cited herein are hereby incorporated by reference.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(I) APPLICANT: Human Genome Sciences, Inc.
9410 Key West Avenue
Rockville, MD 20850
United States of America
APPLICANTS/INVENTORS: Ebner, Reinhard
Yu, Guo-Liang
Ruben, Steven M.

(ii) TITLE OF INVENTION: Apoptosis Inducing Molecule II

(iii) NUMBER OF SEQUENCES: 19

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Sterne, Kessler, Goldstein & Fox, P.L.L.C.
(B) STREET: 1100 New York Ave., Suite 600
(C) CITY: Washington
(D) STATE: DC
(E) COUNTRY: USA
(F) ZIP: 20005-3934

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: TBA
(B) FILING DATE: Herewith
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 60/013,923
(B) FILING DATE: 22 MARCH 1996
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Goldstein, Jorge A.
(B) REGISTRATION NUMBER: 29,021
(C) REFERENCE/DOCKET NUMBER: 1488.065PC01/JAG/EKS/KMT

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 202-371-2600
(B) TELEFAX: 202-371-2540

(2) INFORMATION FOR SEQ ID NO:1:

(I) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1169 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 49..768

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
GAGGTTGAAG GACCCAGGCG TGTCAGCCCT GCTCCAGAGA CCTTGGGC ATG GAG GAG        57
                                                     Met Glu Glu
                                                       1

AGT GTC GTA CGG CCC TCA GTG TTT GTG GTG GAT GGA CAG ACC GAC ATC        105
Ser Val Val Arg Pro Ser Val Phe Val Val Asp Gly Gln Thr Asp Ile
      5                  10                  15

CCA TTC ACG AGG CTG GGA CGA AGC CAC CGG AGA CAG TCG TGC AGT GTG        153
Pro Phe Thr Arg Leu Gly Arg Ser His Arg Arg Gln Ser Cys Ser Val
 20                  25                  30                  35

GCC CGG GTG GGT CTG GGT CTC TTG CTG TTG CTG ATG GGG GCT GGG CTG        201
Ala Arg Val Gly Leu Gly Leu Leu Leu Leu Leu Met Gly Ala Gly Leu
                 40                  45                  50

GCC GTC CAA GGC TGG TTC CTC CTG CAG CTG CAC TGG CGT CTA GGA GAG        249
Ala Val Gln Gly Trp Phe Leu Leu Gln Leu His Trp Arg Leu Gly Glu
             55                  60                  65

ATG GTC ACC CGC CTG CCT GAC GGA CCT GCA GGC TCC TGG GAG CAG CTG        297
Met Val Thr Arg Leu Pro Asp Gly Pro Ala Gly Ser Trp Glu Gln Leu
         70                  75                  80

ATA CAA GAG CGA AGG TCT CAC GAG GTC AAC CCA GCA GCG CAT CTC ACA        345
Ile Gln Glu Arg Arg Ser His Glu Val Asn Pro Ala Ala His Leu Thr
     85                  90                  95

GGG GCC AAC TCC AGC TTG ACC GGC AGC GGG GGG CCG CTG TTA TGG GAG        393
Gly Ala Asn Ser Ser Leu Thr Gly Ser Gly Gly Pro Leu Leu Trp Glu
100                 105                 110                 115

ACT CAG CTG GGC CTG GCC TTC CTG AGG GGC CTC AGC TAC CAC GAT GGG        441
Thr Gln Leu Gly Leu Ala Phe Leu Arg Gly Leu Ser Tyr His Asp Gly
                120                 125                 130

GCC CTT GTG GTC ACC AAA GCT GGC TAC TAC TAC ATC TAC TCC AAG GTG        489
Ala Leu Val Val Thr Lys Ala Gly Tyr Tyr Tyr Ile Tyr Ser Lys Val
            135                 140                 145

CAG CTG GGC GGT GTG GGC TGC CCG CTG GGC CTG GCC AGC ACC ATC ACC        537
Gln Leu Gly Gly Val Gly Cys Pro Leu Gly Leu Ala Ser Thr Ile Thr
        150                 155                 160

CAC GGC CTC TAC AAG CGC ACA CCC CGC TAC CCC GAG GAG CTG GAG CTG        585
His Gly Leu Tyr Lys Arg Thr Pro Arg Tyr Pro Glu Glu Leu Glu Leu
    165                 170                 175
```

TTG GTC AGC CAG CAG TCA CCC TGC GGA CGG GCC ACC AGC AGC TCC CGG 633
Leu Val Ser Gln Gln Ser Pro Cys Gly Arg Ala Thr Ser Ser Ser Arg
180 185 190 195

GTC TGG TGG GAC AGC AGC TTC CTG GGT GGT GTG GTA CAC CTG GAG GCT 681
Val Trp Trp Asp Ser Ser Phe Leu Gly Gly Val Val His Leu Glu Ala
200 205 210

GGG GAG GAG GTG GTC GTC CGT GTG CTG GAT GAA CGC CTG GTT CGA CTG 729
Gly Glu Glu Val Val Val Arg Val Leu Asp Glu Arg Leu Val Arg Leu
215 220 225

CGT GAT GGT ACC CGG TCT TAC TTC GGG GCT TTC ATG GTG TGAAGGAAGG 778
Arg Asp Gly Thr Arg Ser Tyr Phe Gly Ala Phe Met Val
230 235 240

AGCGTGGTGC ATTGGACATG GGTCTGACAC GTGGAGAACT CAGAGGGTGC CTCAGGGGAA 838

AGAAAACTCA CGAAGCAGAG GCTGGGCGTG GTGGCTCTCG CCTGTAATCC CAGCACTTTG 898

GGAGGCCAAG GCAGGCGGAT CACCTGAGGT CAGGAGTTCG AGACCAGCCT GGCTAACATG 958

GCAAAACCCC ATCTCTACTA AAAATACAAA AATTAGCCGG ACGTGGTGGT GCCTGCCTGT 1018

AATCCAGCTA CTCAGGAGGC TGAGGCAGGA TAATTTTGCT TAAACCCGGG AGGCGGAGGT 1078

TGCAGTGAGC CGAGATCACA CCACTGCACT CCAACCTGGG AAACGCAGTG AGACTGTGCC 1138

TCAAAAAAAA AAAAAAAAAA AAAAAAAAAA A 1169

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 240 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Glu Glu Ser Val Val Arg Pro Ser Val Phe Val Val Asp Gly Gln
1 5 10 15

Thr Asp Ile Pro Phe Thr Arg Leu Gly Arg Ser His Arg Arg Gln Ser
20 25 30

Cys Ser Val Ala Arg Val Gly Leu Gly Leu Leu Leu Leu Leu Met Gly
35 40 45

Ala Gly Leu Ala Val Gln Gly Trp Phe Leu Leu Gln Leu His Trp Arg
50 55 60

Leu Gly Glu Met Val Thr Arg Leu Pro Asp Gly Pro Ala Gly Ser Trp

65 70 75 80

Glu Gln Leu Ile Gln Glu Arg Arg Ser His Glu Val Asn Pro Ala Ala
85 90 95

His Leu Thr Gly Ala Asn Ser Ser Leu Thr Gly Ser Gly Gly Pro Leu
100 105 110

Leu Trp Glu Thr Gln Leu Gly Leu Ala Phe Leu Arg Gly Leu Ser Tyr
115 120 125

His Asp Gly Ala Leu Val Val Thr Lys Ala Gly Tyr Tyr Tyr Ile Tyr
130 135 140

Ser Lys Val Gln Leu Gly Gly Val Gly Cys Pro Leu Gly Leu Ala Ser
145 150 155 160

Thr Ile Thr His Gly Leu Tyr Lys Arg Thr Pro Arg Tyr Pro Glu Glu
165 170 175

Leu Glu Leu Leu Val Ser Gln Gln Ser Pro Cys Gly Arg Ala Thr Ser
180 185 190

Ser Ser Arg Val Trp Trp Asp Ser Ser Phe Leu Gly Gly Val Val His
195 200 205

Leu Glu Ala Gly Glu Glu Val Val Val Arg Val Leu Asp Glu Arg Leu
210 215 220

Val Arg Leu Arg Asp Gly Thr Arg Ser Tyr Phe Gly Ala Phe Met Val
225 230 235 240

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 455 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met Gly Leu Ser Thr Val Pro Asp Leu Leu Leu Pro Leu Val Leu Leu
1 5 10 15

Glu Leu Leu Val Gly Ile Tyr Pro Ser Gly Val Ile Gly Leu Val Pro
20 25 30

His Leu Gly Asp Arg Glu Lys Arg Asp Ser Val Cys Pro Gln Gly Lys

35 40 45

Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His Lys
50 55 60

Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp
65 70 75 80

Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser Glu Asn His Leu
85 90 95

Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln Val
100 105 110

Glu Ile Ser Ser Cys Thr Val Asp Arg Asp Thr Val Cys Gly Cys Arg
115 120 125

Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe
130 135 140

Asn Cys Ser Leu Cys Leu Asn Gly Thr Val His Leu Ser Cys Gln Glu
145 150 155 160

Lys Gln Asn Thr Val Cys Thr Cys His Ala Gly Phe Phe Leu Arg Glu
165 170 175

Asn Glu Cys Val Ser Cys Ser Asn Cys Lys Lys Ser Leu Glu Cys Thr
180 185 190

Lys Leu Cys Leu Pro Gln Ile Glu Asn Val Lys Gly Thr Glu Asp Ser
195 200 205

Gly Thr Thr Val Leu Leu Pro Leu Val Ile Phe Phe Gly Leu Cys Leu
210 215 220

Leu Ser Leu Leu Phe Ile Gly Leu Met Tyr Arg Tyr Gln Arg Trp Lys
225 230 235 240

Ser Lys Leu Tyr Ser Ile Val Cys Gly Lys Ser Thr Pro Glu Lys Glu
245 250 255

Gly Glu Leu Glu Gly Thr Thr Thr Lys Pro Leu Ala Pro Asn Pro Ser
260 265 270

Phe Ser Pro Thr Pro Gly Phe Thr Pro Thr Leu Gly Phe Ser Pro Val
275 280 285

Pro Ser Ser Thr Phe Thr Ser Ser Ser Thr Tyr Thr Pro Gly Asp Cys
290 295 300

Pro Asn Phe Ala Ala Pro Arg Arg Glu Val Ala Pro Pro Tyr Gln Gly
305 310 315 320

Ala Asp Pro Ile Leu Ala Thr Ala Leu Ala Ser Asp Pro Ile Pro Asn
325 330 335

Pro Leu Gln Lys Trp Glu Asp Ser Ala His Lys Pro Gln Ser Leu Asp
340 345 350

Thr Asp Asp Pro Ala Thr Leu Tyr Ala Val Val Glu Asn Val Pro Pro
355 360 365

Leu Arg Trp Lys Glu Phe Val Arg Arg Leu Gly Leu Ser Asp His Glu
370 375 380

Ile Asp Arg Leu Glu Leu Gln Asn Gly Arg Cys Leu Arg Glu Ala Gln
385 390 395 400

Tyr Ser Met Leu Ala Thr Trp Arg Arg Arg Thr Pro Arg Arg Glu Ala
405 410 415

Thr Leu Glu Leu Leu Gly Arg Val Leu Arg Asp Met Asp Leu Leu Gly
420 425 430

Cys Leu Glu Asp Ile Glu Glu Ala Leu Cys Gly Pro Ala Ala Leu Pro
435 440 445

Pro Ala Pro Ser Leu Leu Arg
450 455

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 205 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Thr Pro Pro Glu Arg Leu Phe Leu Pro Arg Val Cys Gly Thr Thr
1 5 10 15

Leu His Leu Leu Leu Leu Gly Leu Leu Leu Val Leu Leu Pro Gly Ala
20 25 30

Gln Gly Leu Pro Gly Val Gly Leu Thr Pro Ser Ala Ala Gln Thr Ala
35 40 45

Arg Gln His Pro Lys Met His Leu Ala His Ser Thr Leu Lys Pro Ala
50 55 60

Ala His Leu Ile Gly Asp Pro Ser Lys Gln Asn Ser Leu Leu Trp Arg
65 70 75 80

Ala Asn Thr Asp Arg Ala Phe Leu Gln Asp Gly Phe Ser Leu Ser Asn

85 90 95

Asn Ser Leu Leu Val Pro Thr Ser Gly Ile Tyr Phe Val Tyr Ser Gln
100 105 110

Val Val Phe Ser Gly Lys Ala Tyr Ser Pro Lys Ala Thr Ser Ser Pro
115 120 125

Leu Tyr Leu Ala His Glu Val Gln Leu Phe Ser Ser Gln Tyr Pro Phe
130 135 140

His Val Pro Leu Leu Ser Ser Gln Lys Met Val Tyr Pro Gly Leu Gln
145 150 155 160

Glu Pro Trp Leu His Ser Met Tyr His Gly Ala Ala Phe Gln Leu Thr
165 170 175

Gln Gly Asp Gln Leu Ser Thr His Thr Asp Gly Ile Pro His Leu Val
180 185 190

Leu Ser Pro Ser Thr Val Phe Phe Gly Ala Phe Ala Leu
195 200 205

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 205 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met Thr Pro Pro Glu Arg Leu Phe Leu Pro Arg Val Cys Gly Thr Thr
1 5 10 15

Leu His Leu Leu Leu Leu Gly Leu Leu Leu Val Leu Leu Pro Gly Ala
20 25 30

Gln Gly Leu Pro Gly Val Gly Leu Thr Pro Ser Ala Ala Gln Thr Ala
35 40 45

Arg Gln His Pro Lys Met His Leu Ala His Ser Thr Leu Lys Pro Ala
50 55 60

Ala His Leu Ile Gly Asp Pro Ser Lys Gln Asn Ser Leu Leu Trp Arg
65 70 75 80

Ala Asn Thr Asp Arg Ala Phe Leu Gln Asp Gly Phe Ser Leu Ser Asn
85 90 95

```
Asn Ser Leu Leu Val Pro Thr Ser Gly Ile Tyr Phe Val Tyr Ser Gln
            100                 105                 110

Val Val Phe Ser Gly Lys Ala Tyr Ser Pro Lys Ala Thr Ser Ser Pro
        115                 120                 125

Leu Tyr Leu Ala His Glu Val Gln Leu Phe Ser Ser Gln Tyr Pro Phe
    130                 135                 140

His Val Pro Leu Leu Ser Ser Gln Lys Met Val Tyr Pro Gly Leu Gln
145                 150                 155                 160

Glu Pro Trp Leu His Ser Met Tyr His Gly Ala Ala Phe Gln Leu Thr
                165                 170                 175

Gln Gly Asp Gln Leu Ser Thr His Thr Asp Gly Ile Pro His Leu Val
            180                 185                 190

Leu Ser Pro Ser Thr Val Phe Phe Gly Ala Phe Ala Leu
        195                 200                 205
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 281 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Met Gln Gln Pro Phe Asn Tyr Pro Tyr Pro Gln Ile Tyr Trp Val Asp
1               5                   10                  15

Ser Ser Ala Ser Ser Pro Trp Ala Pro Pro Gly Thr Val Leu Pro Cys
            20                  25                  30

Pro Thr Ser Val Pro Arg Arg Pro Gly Gln Arg Arg Pro Pro Pro Pro
        35                  40                  45

Pro Pro Pro Pro Pro Leu Pro Pro Pro Pro Pro Pro Pro Pro Leu Pro
    50                  55                  60

Pro Leu Pro Leu Pro Pro Leu Lys Lys Arg Gly Asn His Ser Thr Gly
65                  70                  75                  80

Leu Cys Leu Leu Val Met Phe Phe Met Val Leu Val Ala Leu Val Gly
                85                  90                  95

Leu Gly Leu Gly Met Phe Gln Leu Phe His Leu Gln Lys Glu Leu Ala
```

```
                100                 105                 110
Glu Leu Arg Glu Ser Thr Ser Gln Met His Thr Ala Ser Ser Leu Glu
        115                 120                 125

Lys Gln Ile Gly His Pro Ser Pro Pro Pro Glu Lys Lys Glu Leu Arg
    130                 135                 140

Lys Val Ala His Leu Thr Gly Lys Ser Asn Ser Arg Ser Met Pro Leu
145                 150                 155                 160

Glu Trp Glu Asp Thr Tyr Gly Ile Val Leu Leu Ser Gly Val Lys Tyr
                165                 170                 175

Lys Lys Gly Gly Leu Val Ile Asn Glu Thr Gly Leu Tyr Phe Val Tyr
            180                 185                 190

Ser Lys Val Tyr Phe Arg Gly Gln Ser Cys Asn Asn Leu Pro Leu Ser
        195                 200                 205

His Lys Val Tyr Met Arg Asn Ser Lys Tyr Pro Gln Asp Leu Val Met
    210                 215                 220

Met Glu Gly Lys Met Met Ser Tyr Cys Thr Thr Gly Gln Met Trp Ala
225                 230                 235                 240

Arg Ser Ser Tyr Leu Gly Ala Val Phe Asn Leu Thr Ser Ala Asp His
                245                 250                 255

Leu Tyr Val Asn Val Ser Glu Leu Ser Leu Val Asn Phe Glu Glu Ser
            260                 265                 270

Gln Thr Phe Phe Gly Leu Tyr Lys Leu
        275                 280
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GCGGGATCCG GAGAGATGGT CACC 24

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CGCAAGCTTC CTTCACACCA TGAAAGC 27

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GACCGGATCC ATGGAGGAGA GTGTCGTACG GC 32

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CGCAAGCTTC CTTCACACCA TGAAAGC 27

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GCTCCAGGAT CCGCCATCAT GGAGGAGAGT GTCGTACGGC 40

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GACGCGGTAC CGTCCAATGC ACCACGCTCC TTCCTTC 37

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GCTCGGATCC GCCATCATG 19

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 71 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GATGTTCTAG AAAGCGTAGT CTGGGACGTC GTATGGGTAC ACCATGAAAG CCCCGAAGTA 60

AGACCGGGTA C 71

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GCTCCAGGAT CCGCCATCAT GGAGGAGAGT GTCGTACGGC 40

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GACGCGGTAC CGTCCAATGC ACCACGCTCC TTCCTTC 37

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GACGCCCATG GAGGAGGAGA GTGTCGTACG GC 32

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GACCGGATCC CACCATGAAA GCCCCGAAGT AAG 33

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

CGCAAGCTTC CTTCACACCA TGAAAGC 27

Applicant's or agent's file reference number 1486.065PC01	International application No. TBA

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 4, line 1.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet ☐

Name of depositary institution

AMERICAN TYPE CULTURE COLLECTION

Address of depositary institution *(including postal code and country)*

12301 Parklawn Drive
Rockville, Maryland 20852
United States of America

Date of deposit	Accession Number
22 August 1996	ATCC Designation 97689

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet ☐

DNA Plasmid AIM-2

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
☐ This sheet was received with the international application	☐ This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

Form PCT/RO/134 (July 1992)

What Is Claimed Is:

1. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group consisting of:

(a) a nucleotide sequence encoding the AIM II polypeptide having the complete amino acid sequence in Figures 1A-C (SEQ ID NO:2);

(b) a nucleotide sequence encoding the AIM II polypeptide having the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97689;

(c) a nucleotide sequence encoding the AIM II polypeptide extracellular domain;

(d) a nucleotide sequence encoding the AIM II polypeptide transmembrane domain;

(e) a nucleotide sequence encoding the AIM II polypeptide intracellular domain;

(f) a nucleotide sequence encoding a soluble AIM II polypeptide having the extracellular and intracellular domains but lacking the transmembrane domain; and

(g) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e) or (f) above.

2. The nucleic acid molecule of claim 1 wherein said polynucleotide has the complete nucleotide sequence in Figures 1A-C (SEQ ID NO:1).

3. The nucleic acid molecule of claim 1 wherein said polynucleotide has the nucleotide sequence in Figures 1A-C (SEQ ID NO:1) encoding the AIM II polypeptide having the complete amino acid sequence in Figures 1A-C (SEQ ID NO:2).

4. The nucleic acid molecule of claim 1 wherein said polynucleotide has the complete nucleotide sequence of the cDNA clone contained in ATCC Deposit No. 97689.

5. The nucleic acid molecule of claim 1 wherein said polynucleotide has the nucleotide sequence encoding the AIM II polypeptide having the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97689.

6. An isolated nucleic acid molecule comprising a polynucleotide which hybridizes under stringent hybridization conditions to a polynucleotide having a nucleotide sequence identical to a nucleotide sequence in (a), (b), (c), (d), (e), (f) or (g) of claim 1 wherein said polynucleotide which hybridizes does not hybridize under stringent hybridization conditions to a polynucleotide having a nucleotide sequence consisting of only A residues or of only T residues.

7. An isolated nucleic acid molecule comprising a polynucleotide which encodes the amino acid sequence of an epitope-bearing portion of an AIM II polypeptide having an amino acid sequence in (a), (b), (c), (d), (e) or (f) of claim 1.

8. The isolated nucleic acid molecule of claim 7, which encodes an epitope-bearing portion of an AIM II polypeptide selected from the group consisting of: a polypeptide comprising amino acid residues from about 13 to about 20 in Figures 1A-C (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 23 to about 36 in Figures 1A-C (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 69 to about 79 in Figures 1A-C (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 85 to about 94 in Figures 1A-C (SEQ ID NO:2);a polypeptide comprising amino acid residues from about 167 to about 178 in Figures 1A-C

(SEQ ID NO:2);a polypeptide comprising amino acid residues from about 184 to about 196 in Figures 1A-C (SEQ ID NO:2); and a polypeptide comprising amino acid residues from about 221 to about 233 in Figures 1A-C (SEQ ID NO:2).

9. A method for making a recombinant vector comprising inserting an isolated nucleic acid molecule of claim 1 into a vector.

10. A recombinant vector produced by the method of claim 9.

11. A method of making a recombinant host cell comprising introducing the recombinant vector of claim 10 into a host cell.

12. A recombinant host cell produced by the method of claim 11.

13. A recombinant method for producing an AIM II polypeptide, comprising culturing the recombinant host cell of claim 12 under conditions such that said polypeptide is expressed and recovering said polypeptide.

14. An isolated AIM II polypeptide having an amino acid sequence at least 95% identical to a sequence selected from the group consisting of:

(a) the amino acid sequence of the AIM II polypeptide having the complete amino acid sequence in Figures 1A-C (SEQ ID NO:2);

(b) the amino acid sequence of the AIM II polypeptide having the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97689;

(c) the amino acid sequence of the extracellular domain of the AIM II polypeptide;

(d) the amino acid sequence of the transmembrane domain of the AIM II polypeptide;

(e) the amino acid sequence of the intracellular domain of the AIM II polypeptide;

(f) the amino acid sequence of a soluble AIM II polypeptide having the all or part of the extracellular and intracellular domain but lacking the transmembrane domain wherein the extracellular domain; and

(g) the amino acid sequence of an epitope-bearing portion of any one of the polypeptides of (a), (b), (c), (d), (e) or (f).

15. An isolated polypeptide comprising an epitope-bearing portion of the AIM II protein, wherein said portion is selected from the group consisting of: a polypeptide comprising amino acid residues from about 13 to about 20 in Figures 1A-C (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 23 to about 36 in Figures 1A-C (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 69 to about 79 in Figures 1A-C (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 85 to about 94 in Figures 1A-C (SEQ ID NO:2);a polypeptide comprising amino acid residues from about 167 to about 178 in Figures 1A-C (SEQ ID NO:2);a polypeptide comprising amino acid residues from about 184 to about 196 in Figures 1A-C (SEQ ID NO:2); and a polypeptide comprising amino acid residues from about 221 to about 233 in Figures 1A-C (SEQ ID NO:2).

16. An isolated antibody that binds specifically to an AIM II polypeptide of claim 14.

17. A method for the treatment of conditions or diseases selected from the group consisting of lymphadenopathy, autoimmune disease and graft versus host disease comprising administering an effective amount of the polypeptide in (a), (b), (c), (d), (e) or (f) of claim 14 to a patient in need thereof.

18. A method for inhibiting neoplasia comprising administering an effective amount of the polypeptide in (a), (b), (c), (d), (e) or (f) of claim 14 to a patient in need thereof.

19. A method for preventing conditions or diseases selected from the group consisting of septic shock, inflammation, cerebral malaria, activation of the HIV virus, bone resorption, rheumatoid arthritis and cachexia comprising administering an effective amount of an antagonist of the polypeptide of claim 14 to a patient in need thereof.

```
        10                  30                  50
GAGGTTGAAGGACCCAGGCGTGTCAGCCCTGCTCCAGAGACCTTGGGCATGGAGGAGAGT
---------+---------+---------+---------+---------+---------+
                                                 M  E  E  S
        70                  90                 110
GTCGTACGGCCCTCAGTGTTTGTGGTGGATGGACAGACCGACATCCCATTCACGAGGCTG
---------+---------+---------+---------+---------+---------+
V  V  R  P  S  V  F  V  V  D  G  Q  T  D  I  P  F  T  R  L
       130                 150                 170
GGACGAAGCCACCGGAGACAGTCGTGCAGTGTGGCCCGGGTGGGTCTGGGTCTCTTGCTG
---------+---------+---------+---------+---------+---------+
G  R  S  H  R  R  Q  S  C  S  V  A  R  V  G  L  G  L  L  L
       190                 210                 230
TTGCTGATGGGGGCTGGGCTGGCCGTCCAAGGCTGGTTCCTCCTGCAGCTGCACTGGCGT
---------+---------+---------+---------+---------+---------+
L  L  M  G  A  G  L  A  V  Q  G  W  F  L  L  Q  L  H  W  R
       250                 270                 290
CTAGGAGAGATGGTCACCCGCCTGCCTGACGGACCTGCAGGCTCCTGGGAGCAGCTGATA
---------+---------+---------+---------+---------+---------+
L  G  E  M  V  T  R  L  P  D  G  P  A  G  S  W  E  Q  L  I
       310                 330                 350
CAAGAGCGAAGGTCTCACGAGGTCAACCCAGCAGCGCATCTCACAGGGGCCAACTCCAGC
---------+---------+---------+---------+---------+---------+
Q  E  R  R  S  H  E  V  N  P  A  A  H  L  T  G  A  N  S  S
       370                 390                 410
TTGACCGGCAGCGGGGGGCCGCTGTTATGGGAGACTCAGCTGGGCCTGGCCTTCCTGAGG
---------+---------+---------+---------+---------+---------+
L  T  G  S  G  G  P  L  L  W  E  T  Q  L  G  L  A  F  L  R
       430                 450                 470
GGCCTCAGCTACCACGATGGGGCCCTTGTGGTCACCAAAGCTGGCTACTACTACATCTAC
---------+---------+---------+---------+---------+---------+
G  L  S  Y  H  D  G  A  L  V  V  T  K  A  G  Y  Y  Y  I  Y
```

FIG.1A

```
       490               510               530
         .         .         .         .         .         .
TCCAAGGTGCAGCTGGGCGGTGTGGGCTGCCCGCTGGGCCTGGCCAGCACCATCACCCAC
---------+---------+---------+---------+---------+---------+
S  K  V  Q  L  G  G  V  G  C  P  L  G  L  A  S  T  I  T  H
       550               570               590
         .         .         .         .         .         .
GGCCTCTACAAGCGCACACCCCGCTACCCCGAGGAGCTGGAGCTGTTGGTCAGCCAGCAG
---------+---------+---------+---------+---------+---------+
G  L  Y  K  R  T  P  R  Y  P  E  E  L  E  L  L  V  S  Q  Q
       610               630               650
         .         .         .         .         .         .
TCACCCTGCGGACGGGCCACCAGCAGCTCCCGGGTCTGGTGGGACAGCAGCTTCCTGGGT
---------+---------+---------+---------+---------+---------+
S  P  C  G  R  A  T  S  S  S  R  V  W  W  D  S  S  F  L  G
       670               690               710
         .         .         .         .         .         .
GGTGTGGTACACCTGGAGGCTGGGGAGGAGGTGGTCGTCCGTGTGCTGGATGAACGCCTG
---------+---------+---------+---------+---------+---------+
G  V  V  H  L  E  A  G  E  E  V  V  V  R  V  L  D  E  R  L
       730               750               770
         .         .         .         .         .         .
GTTCGACTGCGTGATGGTACCCGGTCTTACTTCGGGGCTTTCATGGTGTGAAGGAAGGAG
---------+---------+---------+---------+---------+---------+
V  R  L  R  D  G  T  R  S  Y  F  G  A  F  M  V  *
       790               810               830
         .         .         .         .         .         .
CGTGGTGCATTGGACATGGGTCTGACACGTGGAGAACTCAGAGGGTGCCTCAGGGGAAAG
---------+---------+---------+---------+---------+---------+
       850               870               890
         .         .         .         .         .         .
AAAACTCACGAAGCAGAGGCTGGGCGTGGTGGCTCTCGCCTGTAATCCCAGCACTTTGGG
---------+---------+---------+---------+---------+---------+
       910               930               950
         .         .         .         .         .         .
AGGCCAAGGCAGGCGGATCACCTGAGGTCAGGAGTTCGAGACCAGCCTGGCTAACATGGC
---------+---------+---------+---------+---------+---------+
       970               990              1010
         .         .         .         .         .         .
AAAACCCCATCTCTAC    TAAAAATACAAAAATTAGCCGGACGTGGTGGTGCCTGCCTGTAA
---------+---------+---------+---------+---------+---------+
```

FIG.1B

```
      1030                  1050                  1070
        .         .         .         .         .         .
TCCAGCTACTCAGGAGGCTGAGGCAGGATAATTTTGCTTAAACCCGGGAGGCGGAGGTTG
---------+---------+---------+---------+---------+---------+
      1090                  1110                  1130
        .         .         .         .         .         .
CAGTGAGCCGAGATCACACCACTGCACTCCAACCTGGGAAACGCAGTGAGACTGTGCCTC
---------+---------+---------+---------+---------+---------+
      1150
        .         .
AAAAAAAAAAAAAAAAAAAAAAAAAAAAA
---------+---------+---------
```

FIG.1C

```
     MTPPE--RLFLPRV--VD------P---G-   Majority
              10        20        30
 1   ME--E--SVVRPSVFVVDGQTDIPFTRLG-   Aim-2.aa
 1   MGLSTVPDLLLPLVLLELLVGIYPSGVIG-   huTNFalpha.prot
 1   MTPPE--RLFLPRV----------------   huTNFbeta.prot
 1   MTPPE--RLFLPRV----------------   huLymphotoxin.prot
 1   MQQPF--NYPYPQIYWVDSSASSPWAPPGT   huFasLigand.prot

     --------------CGTTLH--P--RR--C-  Majority
              40        50        60
26   ----------------------RSHRRQSCS  Aim-2.aa
31   VPHLGDREKRDSVCPQGKYIHPQNNSICCT   huTNFalpha.prot
13   --------------CGTTLH-----------  huTNFbeta.prot
13   --------------CGTTLH-----------  huLymphotoxin.prot
29   VLP-----------CPTSVPRRPGQRRPPPP  huFasLigand.prot

     -----------------------LLL----   Majority
              70        80        90
35   VAR---------------------------   Aim-2.aa
61   KCHKGTYLYNDCPGPGQDTDCRECESGSFT   huTNFalpha.prot
19   -----------------------LLL----   huTNFbeta.prot
19   -----------------------LLL----   huLymphotoxin.prot
49   PPPPPL-------PPPPPPPPLPPLPLPPLK  huFasLigand.prot

     -----------------GLGLLLVLLPGAQ   Majority
```

FIG.2A

```
                     100                 110                 120
 38  -----------------VGLGLLLLMGAGL   Aim-2.aa
 91  ASENHLRHCLSCSKCRKEMGQVEISSCTVD   huTNFalpha.prot
 22  ------------------LGLLLVLLPGAQ   huTNFbeta.prot
 22  ------------------LGLLLVLLPGAQ   huLymphotoxin.prot
 73  KRGNH----------STGLCLLVMFFMVLV   huFasLigand.prot

     --GLPG---------VGL--F-LF-L---L   Majority
                     130                 140                 150
 52  --AVQG---------W----F-LLQLHWRL   Aim-2.aa
121  RDTVCGCRKNQYRHYWSENLFQCFNCSLCL   huTNFalpha.prot
 34  --GLPG---------VGL------------   huTNFbeta.prot
 34  --GLPG---------VGL------------   huLymphotoxin.prot
 93  --ALVG---------LGLGMFQLFHLQKEL   huFasLigand.prot

     -E-V--------------------RTPSAA   Majority
                     160                 170                 180
 66  GEMVT-------------------RLPDGP   Aim-2.aa
151  NGTVHLSCQEKQNTVCTCHAGFFLRENECV   huTNFalpha.prot
 41  -------------------------TPSAA   huTNFbeta.prot
 41  -------------------------TPSAA   huLymphotoxin.prot
112  AELRESTSQ--------------MHTASSL   huFasLigand.prot

     QTARQHPSMELAKSTLKPAAHLIGDPSS--   Majority
```

FIG.2B

```
                 190                 200                 210
77   A G S W E Q L I Q E R R S H E V N P A A H L T G A N S S L T   Aim-2.aa
181  S C S N C K K S L E C T K L C L P Q I E N V K G T E D S G T   huTNFalpha.prot
46   Q T A R Q H P K M H L A H S T L K P A A H L I G D P S K - -   huTNFbeta.prot
46   Q T A R Q H P K M H L A H S T L K P A A H L I G D P S K - -   huLymphotoxin.prot
128  E K Q I G H P S P P P E K K E L R K V A H L T G K S N S - -   huFasLigand.prot

     - Q N - P L L - - - - - - - - - - - - - - - - - - - - - W E   Majority
                 220                 230                 240
107  G S G G P L L - - - - - - - - - - - - - - - - - - - - - W E   Aim-2.aa
211  T V L L P L V I F F G L C L L S L L F I G L M Y R Y Q R W K   huTNFalpha.prot
74   - Q N - S L L - - - - - - - - - - - - - - - - - - - - - W R   huTNFbeta.prot
74   - Q N - S L L - - - - - - - - - - - - - - - - - - - - - W R   huLymphotoxin.prot
156  - R S M P L E - - - - - - - - - - - - - - - - - - - - - W E   huFasLigand.prot

     A N L G R A F - - - - - - - - - - - - - - - - - - - L Q D G   Majority
                 250                 260                 270
116  T Q L G L A F - - - - - - - - - - - - - - - - - - - L R - G   Aim-2.aa
241  S K L Y S I V C G K S T P E K E G E L E G T T T K P L A P N   huTNFalpha.prot
81   A N T D R A F - - - - - - - - - - - - - - - - - - - L Q D G   huTNFbeta.prot
81   A N T D R A F - - - - - - - - - - - - - - - - - - - L Q D G   huLymphotoxin.prot
164  D T Y G I V L - - - - - - - - - - - - - - - - - - - L - S G   huFasLigand.prot

     F S L S N G S L V V P T S G I Y F V Y S Q V V F S G K A Y S   Majority
```

FIG.2C

```
                                          280                                     290                                     300
126  L S Y H D G A L V V T K A G Y Y Y I Y S K V Q L G G V G C P   Aim-2.aa
271  P S F S P T P G F T P T L G F S P V P S S T F T S S S T Y T   huTNFalpha.prot
92   F S L S N N S L L V P T S G I Y F V Y S Q V V F S G K A Y S   huTNFbeta.prot
92   F S L S N N S L L V P T S G I Y F V Y S Q V V F S G K A Y S   huLymphotoxin.prot
174  V K Y K K G G L V I N E T G L Y F V Y S K V Y F R G Q S C N   huFasLigand.prot

     P G A X S S P L Y L A H E V Q L R S S Q Y P F H V P L L S S   Majority
                                          310                                     320                                     330
156  L G L A S T - - - I T H G L Y K R T P R Y P E E L E L L V S   Aim-2.aa
301  P G D C P N F A A P R R E V - - - A P P Y Q G A D P I L A T   huTNFalpha.prot
122  P K A P S S P L Y L A H E V Q L F S S Q Y P F H V P L L S S   huTNFbeta.prot
122  P K A P S S P L Y L A H E V Q L F S S Q Y P F H V P L L S S   huLymphotoxin.prot
204  - - - - - - - N L P L S H K V Y M R N S K Y P Q D L V M M E G   huFasLigand.prot

     Q K M V Y - - - P G L Q E P W L D S S Y L G A A F Q L T Q G   Majority
                                          340                                     350                                     360
183  Q Q S P C G R A T S S S R V W W D S S F L G G V V H L E A G   Aim-2.aa
328  A - L A S D P I P N P L Q K W E D S A H K P Q S L D T D D P   huTNFalpha.prot
152  Q K M V Y - - - P G L Q E P W L H S M Y H G A A F Q L T Q G   huTNFbeta.prot
152  Q K M V Y - - - P G L Q E P W L H S M Y H G A A F Q L T Q G   huLymphotoxin.prot
228  K M M S Y - - - C T T G Q M W A R S S Y L G A V F N L T S A   huFasLigand.prot

     D Q L S V H V D G I P L L V L S E S T - V F F - - - - - - - -   Majority
```

FIG.2D

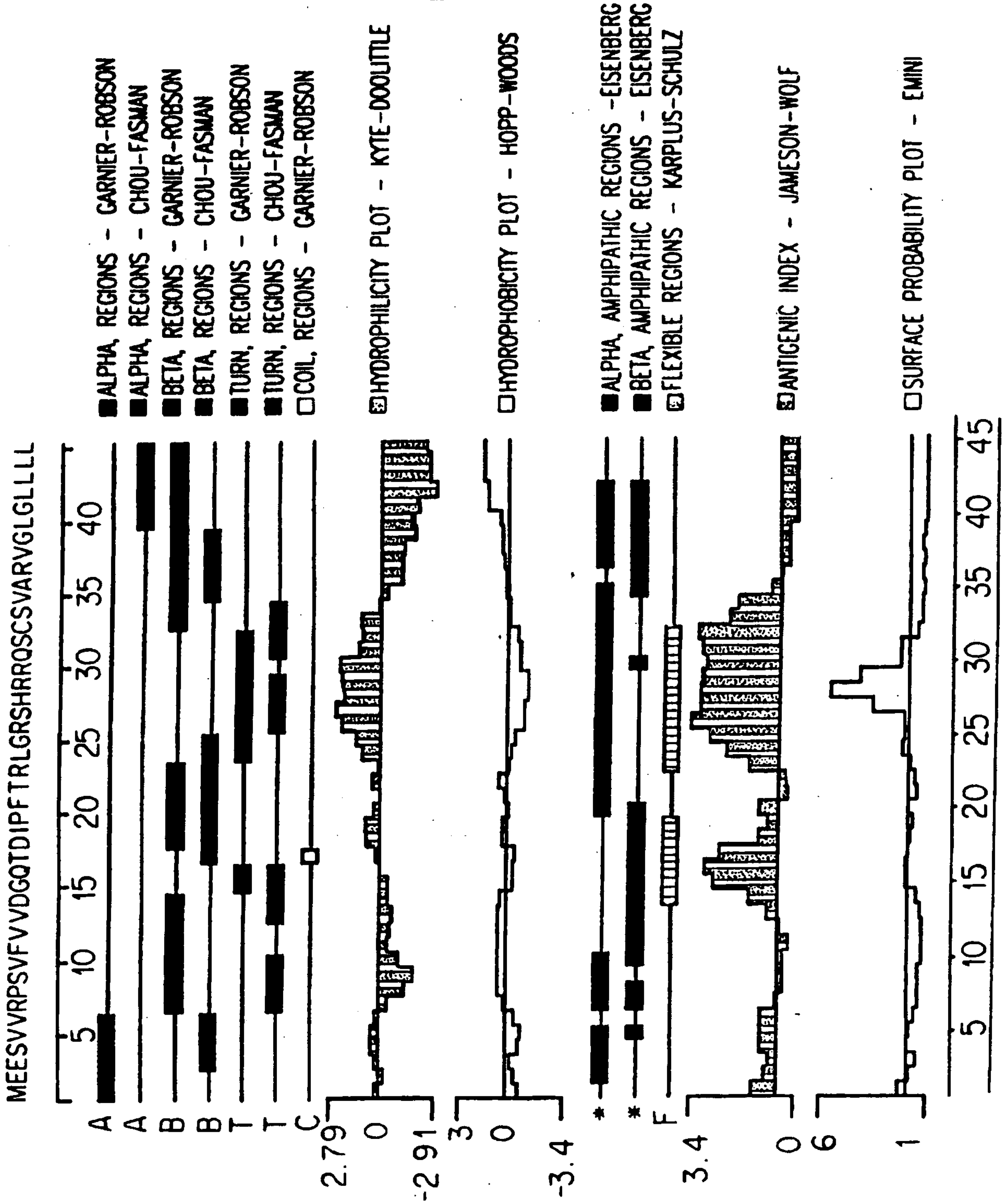
```
                      370                 380                 390
213  E E V V V R V L D E R L V R L R D G T R S Y F - - - - - - -   Aim-2.aa
357  A T L Y A V V E N V P P L R W K E F V R R L G L S D H E I D   huTNFalpha.prot
179  D Q L S T H T D G I P H L V L S P S T - V F F - - - - - - -   huTNFbeta.prot
179  D Q L S T H T D G I P H L V L S P S T - V F F - - - - - - -   huLymphotoxin.prot
255  D H L Y V N V S E L S L V N F E E S Q - T F F - - - - - - -   huFasLigand.prot

     - - - - - - - - - - - - G A F A - L - - - - - - - - - - - -   Majority
                      400                 410                 420
236  - - - - - - - - - - - - G A F M V                             Aim-2.aa
387  R L E L Q N G R C L R E A Q Y S M L A T W R R R T P R R E A   huTNFalpha.prot
201  - - - - - - - - - - - - G A F A - L                           huTNFbeta.prot
201  - - - - - - - - - - - - G A F A - L                           huLymphotoxin.prot
277  - - - - - - - - - - - - G L Y K - L                           huFasLigand.prot

     - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   Majority
                      430                 440                 450
241                                                                Aim-2.aa
417  T L E L L G R V L R D M D L L G C L E D I E E A L C G P A A   huTNFalpha.prot
205                                                                huTNFbeta.prot
205                                                                huLymphotoxin.prot
281                                                                huFasLigand.prot

     - - - - - - - - - -                                           Majority
```

FIG.2E

241		Aim-2.aa
447	L P P A P S L L R	huTNFalpha.prot
205		huTNFbeta.prot
205		huLymphotoxin.prot
281		huFasLigand.prot

FIG.2F

FIG.3A


MEESVVRPSVFVVDGQTDIPFTRLGRSHRRQSCSVARVGLGLLLL
ALPHA, REGIONS - GARNIER-ROBSON
ALPHA, REGIONS - CHOU-FASMAN
BETA, REGIONS - GARNIER-ROBSON
BETA, REGIONS - CHOU-FASMAN
TURN, REGIONS - GARNIER-ROBSON
TURN, REGIONS - CHOU-FASMAN
COIL, REGIONS - GARNIER-ROBSON
HYDROPHILICITY PLOT - KYTE-DOOLITTLE
HYDROPHOBICITY PLOT - HOPP-WOODS
ALPHA, AMPHIPATHIC REGIONS -EISENBERG
BETA, AMPHIPATHIC REGIONS - EISENBERG
FLEXIBLE REGIONS - KARPLUS-SCHULZ
ANTIGENIC INDEX - JAMESON-WOLF
SURFACE PROBABILITY PLOT - EMINI
A
A
B
B
T
T
C
2.79
0
-2.91
3
0
-3.4
*
*
F
3.4
0
6
1
5
10
15
20
25
30
35
40
45

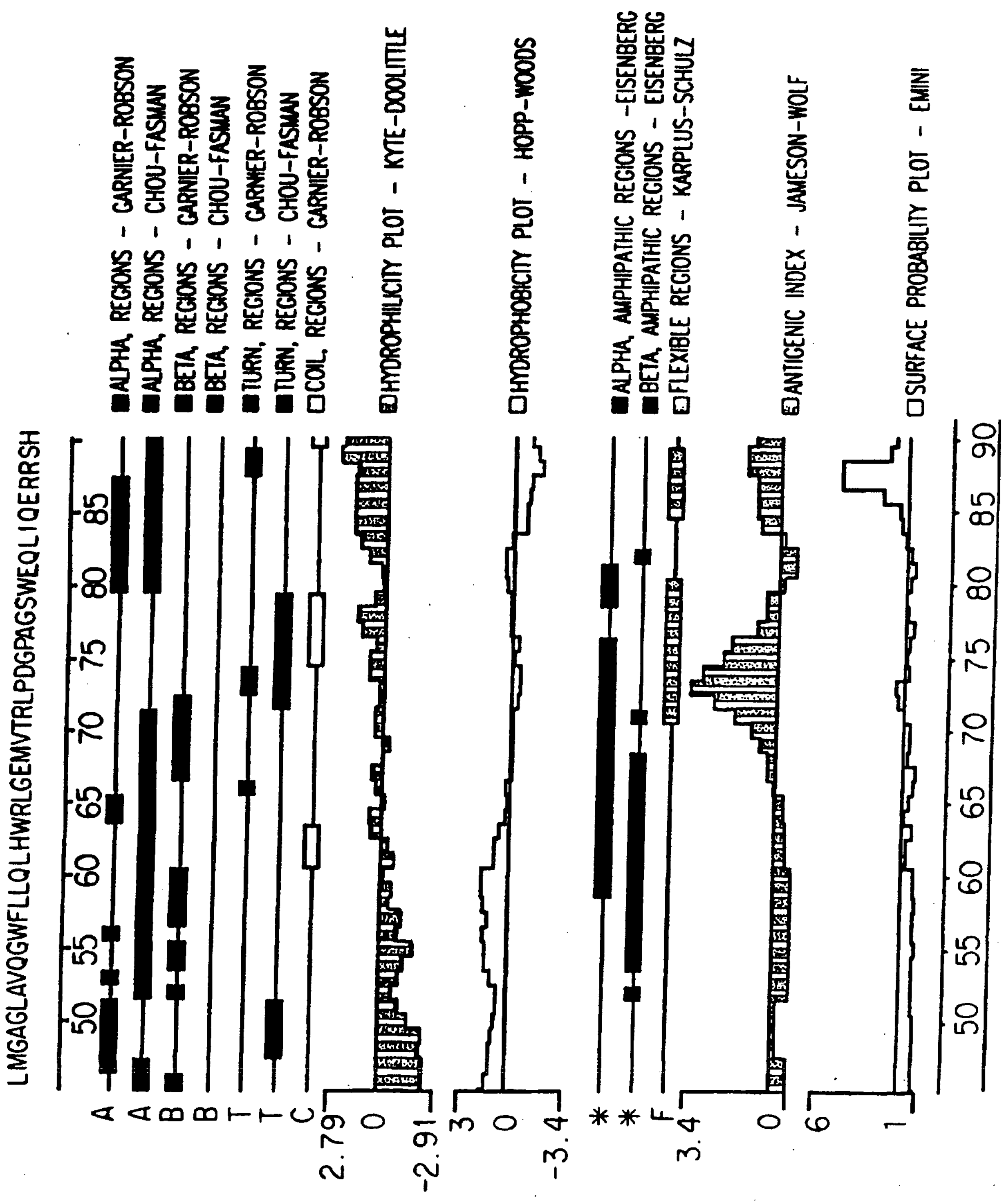

LMGAGLAVQGWFLLQLHWRLGEMVTRLPDGPAGSWEQLIQERRSH
50 55 60 65 70 75 80 85 90
A A B B T T C
ALPHA, REGIONS - GARNIER-ROBSON
ALPHA, REGIONS - CHOU-FASMAN
BETA, REGIONS - GARNIER-ROBSON
BETA, REGIONS - CHOU-FASMAN
TURN, REGIONS - GARNIER-ROBSON
TURN, REGIONS - CHOU-FASMAN
COIL, REGIONS - GARNIER-ROBSON
2.79 0 -2.91
HYDROPHILICITY PLOT - KYTE-DOOLITTLE
3 0 -3.4
HYDROPHOBICITY PLOT - HOPP-WOODS
* * F
ALPHA, AMPHIPATHIC REGIONS -EISENBERG
BETA, AMPHIPATHIC REGIONS - EISENBERG
FLEXIBLE REGIONS - KARPLUS-SCHULZ
3.4 0
ANTIGENIC INDEX - JAMESON-WOLF
6 1
SURFACE PROBABILITY PLOT - EMINI

FIG.3B

FIG.3C

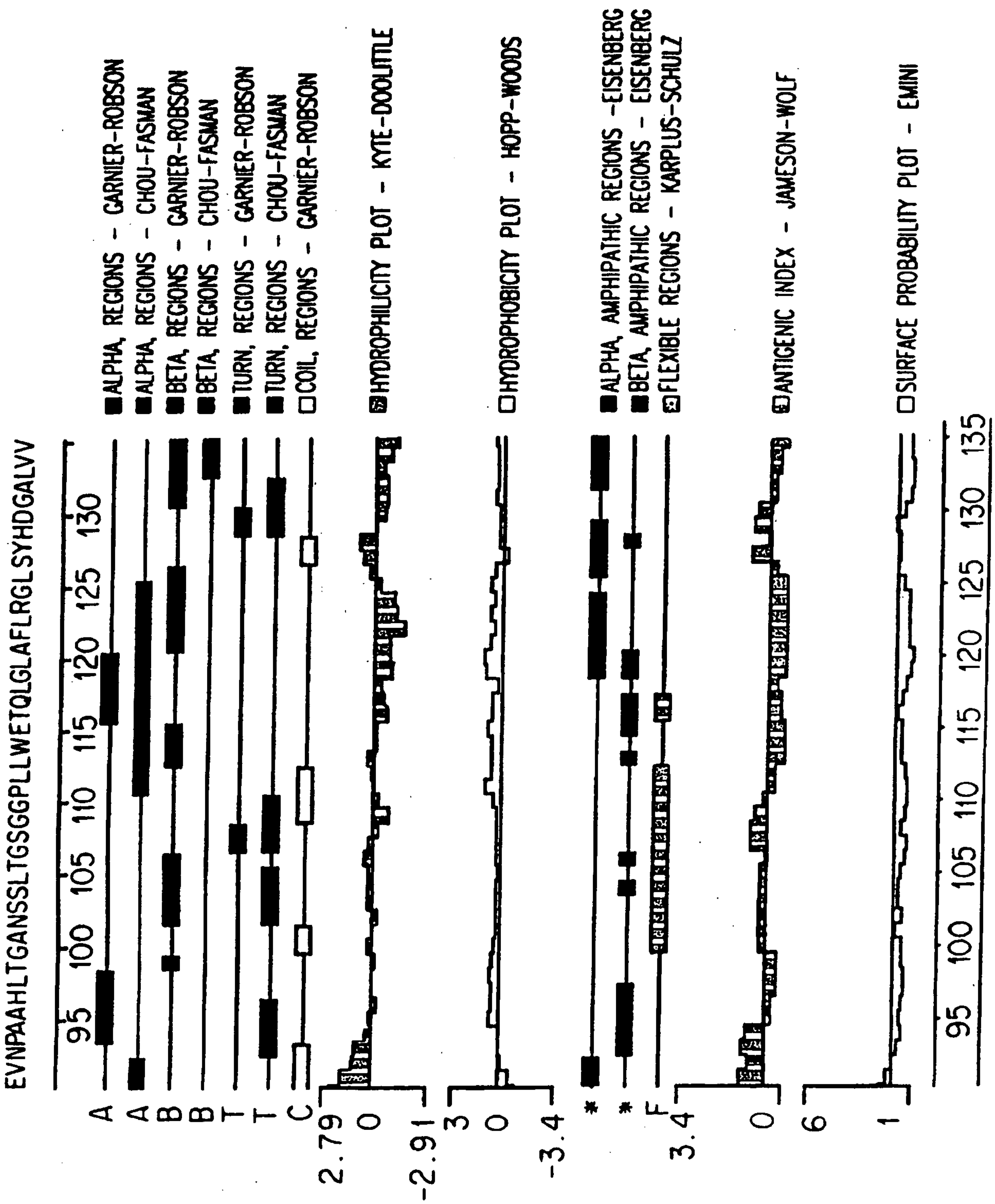
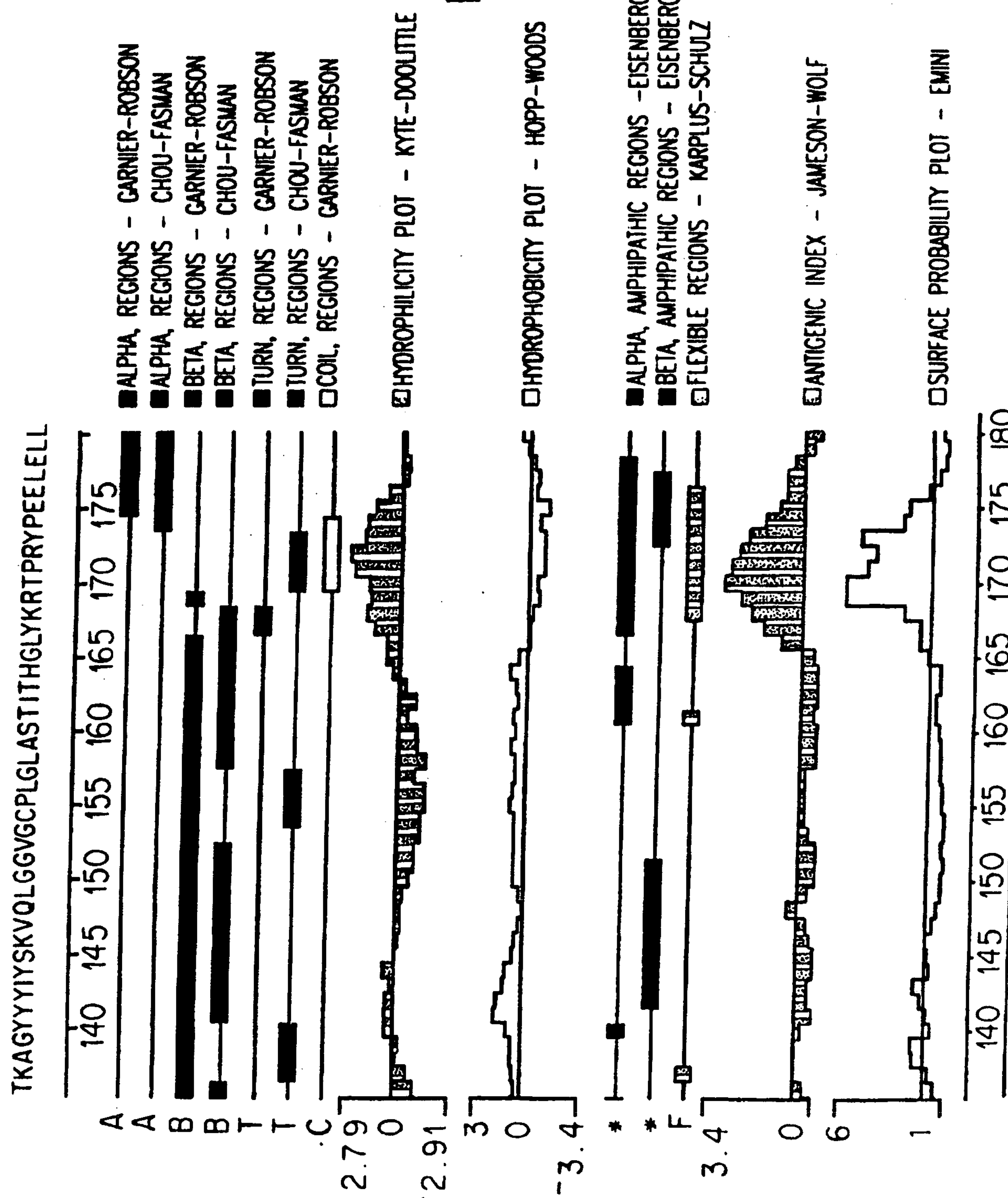

EVNPAAHLTGANSSLTGSGGPLLWETQLGLAFLRGLSYHDGALVV
ALPHA, REGIONS - GARNIER-ROBSON
ALPHA, REGIONS - CHOU-FASMAN
BETA, REGIONS - GARNIER-ROBSON
BETA, REGIONS - CHOU-FASMAN
TURN, REGIONS - GARNIER-ROBSON
TURN, REGIONS - CHOU-FASMAN
COIL, REGIONS - GARNIER-ROBSON
HYDROPHILICITY PLOT - KYTE-DOOLITTLE
HYDROPHOBICITY PLOT - HOPP-WOODS
ALPHA, AMPHIPATHIC REGIONS - EISENBERG
BETA, AMPHIPATHIC REGIONS - EISENBERG
FLEXIBLE REGIONS - KARPLUS-SCHULZ
ANTIGENIC INDEX - JAMESON-WOLF
SURFACE PROBABILITY PLOT - EMINI
A
A
B
B
T
T
C
2.79
0
-2.91
3
0
-3.4
*
*
F
3.4
0
6
1
95
100
105
110
115
120
125
130
135

FIG.3D



TKAGYYIYSKVQLGGVGCPLGLASTITHGLYKRTPRYPEELELL
ALPHA, REGIONS - GARNIER-ROBSON
ALPHA, REGIONS - CHOU-FASMAN
BETA, REGIONS - GARNIER-ROBSON
BETA, REGIONS - CHOU-FASMAN
TURN, REGIONS - GARNIER-ROBSON
TURN, REGIONS - CHOU-FASMAN
COIL, REGIONS - GARNIER-ROBSON
HYDROPHILICITY PLOT - KYTE-DOOLITTLE
HYDROPHOBICITY PLOT - HOPP-WOODS
ALPHA, AMPHIPATHIC REGIONS - EISENBERG
BETA, AMPHIPATHIC REGIONS - EISENBERG
FLEXIBLE REGIONS - KARPLUS-SCHULZ
ANTIGENIC INDEX - JAMESON-WOLF
SURFACE PROBABILITY PLOT - EMINI
A
A
B
B
T
T
C
2.79
0
-2.91
3
0
-3.4
*
*
F
3.4
0
6
1
140
145
150
155
160
165
170
175
180

FIG.3E

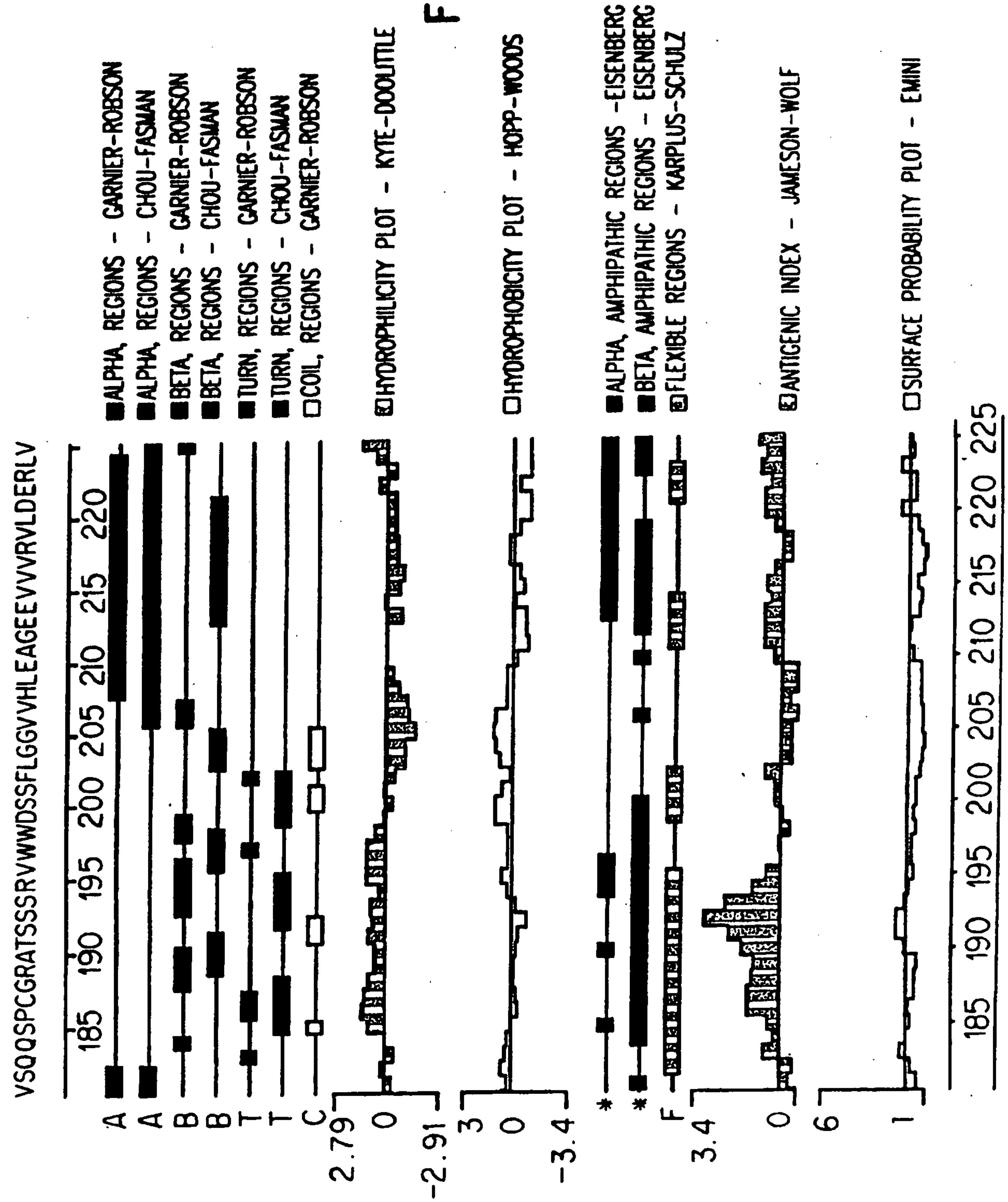
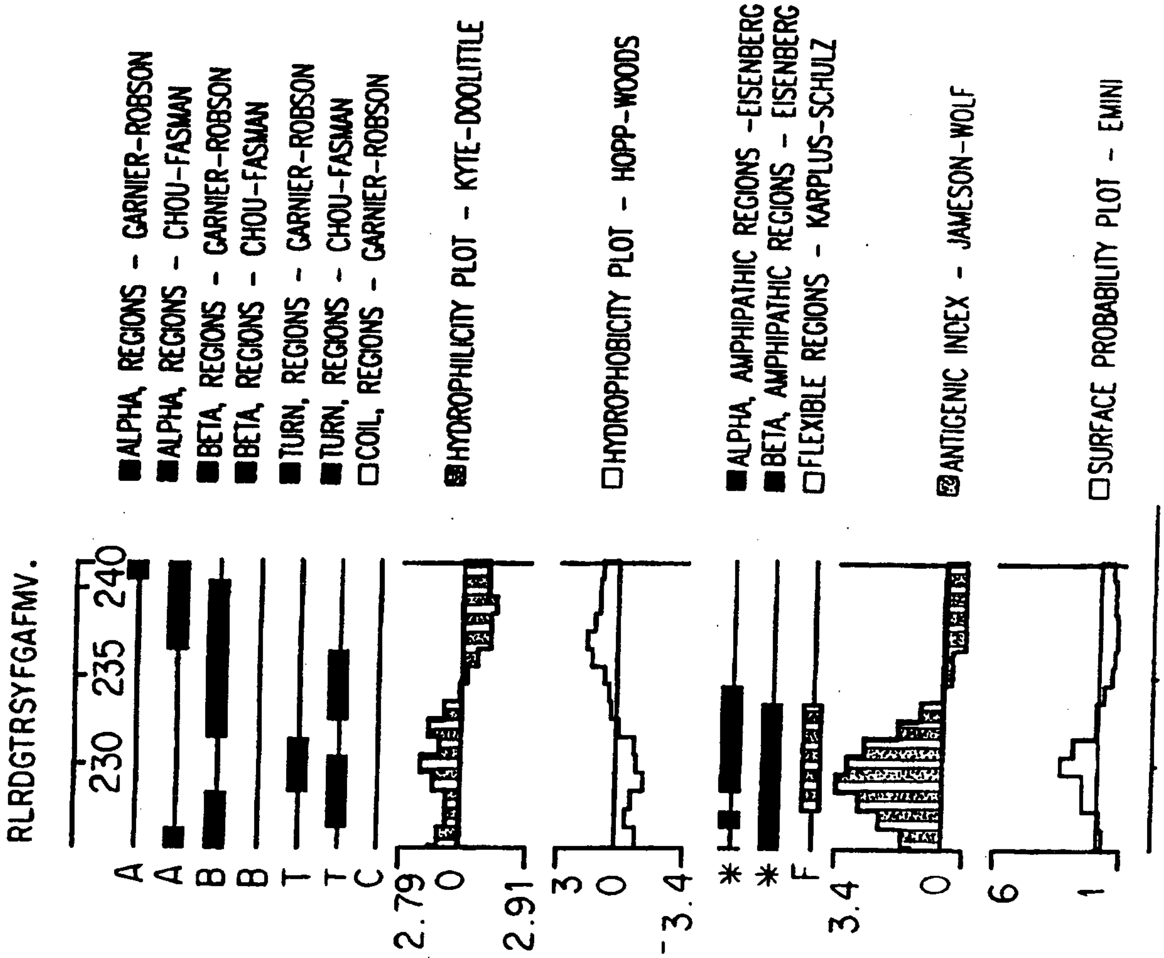

VSQQSPCGRATSSSRVWWDSSFLGGVVHLEAGEEVVVRVLDERLV
185 190 195 200 205 210 215 220 225
ALPHA, REGIONS - GARNIER-ROBSON
ALPHA, REGIONS - CHOU-FASMAN
BETA, REGIONS - GARNIER-ROBSON
BETA, REGIONS - CHOU-FASMAN
TURN, REGIONS - GARNIER-ROBSON
TURN, REGIONS - CHOU-FASMAN
COIL, REGIONS - GARNIER-ROBSON
HYDROPHILICITY PLOT - KYTE-DOOLITTLE
HYDROPHOBICITY PLOT - HOPP-WOODS
ALPHA, AMPHIPATHIC REGIONS -EISENBERG
BETA, AMPHIPATHIC REGIONS - EISENBERG
FLEXIBLE REGIONS - KARPLUS-SCHULZ
ANTIGENIC INDEX - JAMESON-WOLF
SURFACE PROBABILITY PLOT - EMINI
A
A
B
B
T
T
C
2.79
0
-2.91
3
0
-3.4
*
*
F
3.4
0
6
1

FIG.3F


RLRDGTRSYFGAFMV.
230 235 240
ALPHA, REGIONS - GARNIER-ROBSON
ALPHA, REGIONS - CHOU-FASMAN
BETA, REGIONS - GARNIER-ROBSON
BETA, REGIONS - CHOU-FASMAN
TURN, REGIONS - GARNIER-ROBSON
TURN, REGIONS - CHOU-FASMAN
COIL, REGIONS - GARNIER-ROBSON
HYDROPHILICITY PLOT - KYTE-DOOLITTLE
HYDROPHOBICITY PLOT - HOPP-WOODS
ALPHA, AMPHIPATHIC REGIONS -EISENBERG
BETA, AMPHIPATHIC REGIONS - EISENBERG
FLEXIBLE REGIONS - KARPLUS-SCHULZ
ANTIGENIC INDEX - JAMESON-WOLF
SURFACE PROBABILITY PLOT - EMINI
A
A
B
B
T
T
C
2.79
0
-2.91
3
0
-3.4
*
*
F
3.4
0
6
1
230 235

INTERNATIONAL SEARCH REPORT	International application No. PCT/US96/16966

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.1, 24.33; 435/320.1, 69.1; 530/350, 351; 424/130.1; 514/2, 1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Dialog, APS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	TAKEDA et al. Rapid Acceleration of Neutrophil Apoptosis by Tumor Necrosis Factor-alpha. International Immunology. 1993, Vol.5, No.6, pages 691-694.	1-19
A	GRUSS et al. Structural and Biological Features of the TNF Receptor and TNF Ligand Superfamilies: Interactive Signals in the Pathobiology of Hodgkins Disease. Annals of Oncology. 1996, Vol.7, Suppl. 4, S19-S26.	1-19
A	GRUSS et al. CD30 Ligand, a Member of the TNF Ligand Superfamily, with Growth and Activation Control for CD30+ Lymphoid and Lymphoma Cells. Leukemia and Lymphoma. 1996, Vol. 20, pages 397-409.	1-19

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 FEBRUARY 1997	12 MAR 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer ENRIQUE D. LONGTON Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GRUSS et al. Tumor Necrosis Factor Ligand Superfamily: Involvement in the Pathology of Malignant Lymphomas. Blood.15 June 1995, Vol.85, No.12, pages 3378-3404.	1-19
A	GRUSS et al. The TNF Ligand Superfamily and its Relevance for Human Diseases. Cytokines and Molecular Therapy. 1995, Vol.1, pages 75-105.	1-19

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

C07H 21/04; C12N 15/00; C12P 21/00; C07K 14/00; A61K 39/395, 38/00, 31/00

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

536/23.1, 24.33; 435/320.1, 69.1; 530/350, 351; 424/130.1; 514/2, 1